

**INFLUENCE OF SITE FACTORS AND VASCULAR
CONDUCTIVITY ON THE DEVELOPMENT OF
PRO CERUM ROOT DISEASE**

by

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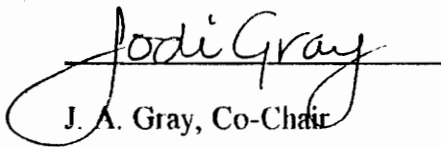
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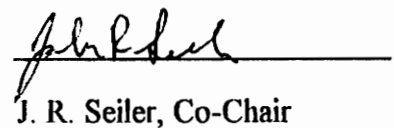
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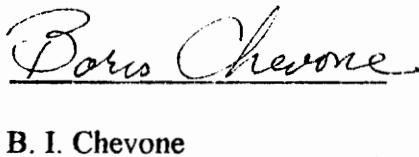
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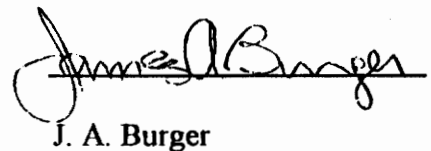
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(ABSTRACT)

Procerum root disease (PRD) is a serious problem in eastern white pine (*Pinus strobus* L.) Christmas tree plantations in Virginia. Procerum root disease is caused by *Leptographium procerum* (Kendr.) Wingf. which is believed to be transmitted by the pales weevil *Hylobius pales* (Herbst). Symptoms of procerum root disease include reduced shoot elongation, reduced leaf conductance, low photosynthetic activity, low pre-dawn water potential and chlorosis of foliage. Resinous occlusion of the sapwood at the root collar is the likely cause of the suite of symptoms that resemble water stress. Increased incidence of PRD has been associated with trees growing in poorly drained soils in low lying areas. Two studies were undertaken to explore the relationship of site factors and vascular conductivity of sapwood to the expression of PRD symptoms in *P. strobus*. In the first study, plots were established in a variety of drainage classes in two Christmas tree plantations. Leaf conductance was monitored periodically in conjunction with measurements of soil factors to assess the role of abiotic factors on foliar symptom expression. At the termination of the field monitoring, trees were harvested and three vascular disease severity variables were measured: hydraulic conductivity of sapwood, percent basal occlusion and sapwood moisture content. These disease severity variables describe the permeability of sapwood to water and the relative

hydration of the sapwood. Reduced leaf conductance was associated with reduced stem hydraulic conductivity, reduced sapwood moisture content and increased basal occlusion. Increased vascular disease severity and foliar symptom expression were associated with increased soil moisture content and several other factors that relate to soil moisture retention (percent slope, total porosity, textural class and bulk density). The second study was initiated to study the development of PRD in artificially inoculated *P. strobus* seedlings exposed to three soil water classes: droughty, optimum and saturated. Seedlings in the optimum soil water class exhibited the greatest biomass accumulation and shoot elongation, while seedlings in droughty showed the least. No negative effects of *L. procerum* inoculation and no symptoms of PRD were observed eight months after inoculation regardless of the soil water class.

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TABLE OF CONTENTS

	Page
TITLE.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vi
LIST OF FIGURES.....	viii
INTRODUCTION.....	1
CHAPTER 1. Literature Review.....	4
CHAPTER 2. Influence of Site Factors on the Physiology of Eastern White Pine (<i>Pinus strobus</i> L.) with Procerum Root Disease in Christmas Tree Plantations.....	20
CHAPTER 3. Influence of Soil Moisture on the Development of Procerum Root Disease Symptoms in Eastern White Pine (<i>Pinus strobus</i> L.) Seedlings.....	51
LITERATURE CITED.....	78
VITA.....	89

LIST OF TABLES

Table		Page
2.1	Topographic and soil characteristics of plantations and plots used in study.....	23
2.2	Mean soil characteristics for plantations and plots used in study.....	26
2.3	Influence of disease class on stem hydraulic properties.....	32
2.4	Comparative affects of Lp, percent basal occlusion, and percent sapwood moisture content of eastern white pine using linear regression analysis.....	34
2.5	Correlation coefficients (r) among all disease severity variables.....	35
2.6	Analysis of weed control methods on soil moisture at 10 cm, 20 cm and on leaf conductance.....	42
2.7	Linear regression summary of independent site variables affecting leaf conductance.....	44
2.8	Linear regression summary of independent site variables affecting Lp.....	45
2.9	Linear regression summary of independent site variables affecting basal occlusion (%).....	46
2.10	Linear regression summary of independent site variables affecting sapwood moisture content (%).....	47
3.1	Analysis of variance of seedling physiology variables 36 weeks after inoculation.....	60
3.2	Water relations of 2-0 eastern white pine seedlings 36 weeks after inoculation.....	61

Table	Page
3.3	Analysis of variance of seedling physiology variables after droughty treatment was rewatered and daily watering of saturated treatment was suspended.....62
3.4	Water relations of (2-0) eastern white pine seedlings across soil water treatments after droughty treatment was re-watered and daily watering of the saturated treatment was suspended.....63
3.5	Analysis of variance of seedling xylem hydraulic conductivity.....65
3.6	Xylem hydraulic conductivity of (2-0) eastern white pine seedlings as influenced by inoculation with <i>L. procerum</i> and soil water condition.....66
3.7	Analysis of variance of seedling biomass and morphology variables.....69
3.8	Biomass and morphology of 2-0 eastern white pine seedlings as influenced by <i>L. procerum</i> inoculation and soil water treatments.....71

LIST OF FIGURES

Figure	Page
2.1.	Stomatal Conductance of healthy and diseased eastern white pine over the duration of the field study.....31
2.2. a, b, c.	Effect of Lp, percent basal occlusion and sapwood moisture content on leaf conductance (\pm 95% C.I.) in <i>P. strobus</i>36
2.3. a, b, c.	Effect of Lp, percent basal occlusion and sapwood moisture content on leaf conductance (\pm 95% C.I) in <i>P. strobus</i> on day 273...37
2.4.	Relationship between basal occlusion (%) and Lp (\pm 95% C.I.) in <i>P. strobus</i>40
2.5. a, b.	Relationship between Lp and basal occlusion (%), and sapwood moisture content (\pm 95% C.I.) in <i>P. strobus</i>41
2.6. a, b, c, d.	Relationship between disease severity variables and soil moisture at 20 cm normalized by date (\pm 95% C.I.).....48
3.1	Mean cumulative shoot elongation of 2-0 <i>P. strobus</i> following inoculation treatments. Elongation in the <i>L. procerum</i> and MEA (control) treatments was found to be significantly different using Tukey's HSD test (α =0.05) at dates 83, 96, and 107, but not date 144.....67
3.2	Mean cumulative shoot elongation of 2-0 <i>P. strobus</i> as influenced by watering treatment. Elongation in the droughty, optimum and saturated moisture treatments was found to be significantly different at all measurement dates using Tukey's HSD test (α =0.05).....68

Introduction

Procerum root disease (PRD) is caused by the deuteromycete fungus *Leptographium procerum* (Kendr.) Wingf., formerly *Verticicladiella procera* (Kendr.) and affects eastern white pine (*Pinus strobus* L.) and Scots pine (*Pinus sylvestris* L.). In the eastern United States, PRD has become a serious disease problem during the last decade affecting *P. strobus* Christmas trees as well as trees in the urban landscape (Lackner and Alexander 1982, 1984). Christmas tree production in Virginia has become increasingly popular with 1.7 million trees harvested per annum (Nichols and Torbert 1989). A large proportion of these trees are white pine, making PRD a potentially economically important tree disease. In plantations where PRD occurs, annual losses are typically 1-3%, with cumulative losses of 20% over a rotation. In severe cases, losses as high as 50% have been recorded (Anderson and Alexander 1979). Incidence of PRD in Virginia Christmas tree farms is currently at epidemic levels (Carlson 1994). *Leptographium procerum* is most commonly isolated from pines, though the fungus has also been isolated from other conifer species including: Fraser fir (*Abies fraseri*), Douglas-fir (*Pseudotsuga menziesii*), and Norway spruce (*Picea abies*) (Alexander et al. 1988).

The initial foliar symptoms of PRD in white pine include mild chlorosis, delayed bud break, and inhibited shoot elongation relative to healthy cohorts (Anderson and Alexander 1979, Alexander 1980, Carlson 1994). Resin exudation from the lower stem, basal irregularities due to localized cambium death, and basal swelling may accompany the initial foliar symptoms (Alexander et al. 1988, Carlson 1994). As the disease progresses, wilting and marked chlorosis of the foliage are observed. Eventually, the foliage develops a reddish color indicating mortality (Carlson 1994). Observations of severe foliar symptoms are usually found in association with substantial resin soaking. Resin soaked sapwood was found to be significantly less permeable to water than asymptomatic sapwood (Horner 1985). In white pine plantations aged 6 to 10 years, resin soaking is a typical expression of PRD while black staining is rare (Carlson 1994).

To quantify basal occlusion Carlson (1994) placed basal stem segments in fast green dye and allowed them to absorb dye for several hours. The segments were cross sectioned with a band saw to reveal green stained conducting areas and unstained occluded areas. A disease severity scale was developed using the percent of basal area that was occluded. Xylem occlusion by resin infiltration has been associated with low pre-dawn water potentials (PDWP) (Carlson 1994). It was noted that some moderately to severely diseased trees had PDWP levels consistent with those of healthy trees. Other trees exhibited low PDWP with only low to moderate disease severity ratings. Carlson (1994) suggested that these observations may be due to variations in soil moisture. With the exception of resin soaking, the symptoms of PRD in white pine are indistinguishable from water stress symptoms. It is probable that any factor that reduces the ability of a tree to extract soil water and transport it to the leaves will hasten the onset of PRD symptoms.

The focus of this research was to explore the relationship of soil moisture and vascular conductivity to the expression of PRD symptoms and disease severity in white pine. Leaf conductance was used as a measure of symptom expression and disease severity was indexed by three vascular variables: hydraulic conductivity of the basal stem, percent basal occlusion and sapwood moisture content. Leaf conductance measured periodically over the course of the growing season in conjunction with volumetric soil water content measures in the immediate vicinity of each study tree and allowed direct comparison of the foliar symptom expression and soil moisture. Volumetric water content of the soil in the immediate vicinity of each study tree was monitored for direct comparison with foliar symptom expression measures. The climate of southwest Virginia is typified by moist spring seasons with gradually drying as the summer progresses, culminating in a dry late summer/early fall season. These conditions were compared to a greenhouse "dry-down" experiment where effects of water stress magnify as the experiment continues. At the end of the season, xylem conductivity of a basal stem segment from each tree was measured and the relation between soil moisture properties, xylem conductivity and PRD disease expression was analyzed.

General Objectives

To evaluate the influence of site factors and vascular conductivity of water on the development of procerum root disease (PRD) (pathogenic agent: *Leptographium procerum*) symptoms in plantation grown eastern white pine Christmas trees and artificially inoculated seedlings grown under greenhouse conditions.

Specific Objectives

1. To determine whether the expression of foliar PRD symptoms in eastern white pine is influenced by the degree of vascular permeability of the lower stem just above the root collar.
2. To determine whether the expression of PRD symptoms and disease severity in eastern white pine are influenced by microsite soil factors.
3. To isolate and determine the influence of soil moisture on symptom expression and disease severity using artificially inoculated *P. strobus* seedlings.

Chapter 1

Literature Review

The Pathogen

Leptographium procerum was first described by Kendrick (1962) as *Verticicladiella procera*. The fungus was later reclassified to the genus *Leptographium* (Lagerb. and Melin) based on greater similarity in conidial development with other members of the genus (Wingfield 1985). *Leptographium procerum* is a member of the *Leptographium* complex which includes mostly wood staining fungi (blue and black staining) and is characterized by the presence of "darkly pigmented mononematous conidiospores" (Kendrick 1962) borne in mucilaginous drops supported by dark colored conidiophores (Cobb 1988). *Leptographium procerum* is an imperfect fungus whose sexual stage has never been found. It is predicted that its sexual anamorph would be classified in the genus *Ophiostoma* (bluestain fungi) based on the presence of cellulose in the cell walls of *L. procerum* (Horner and Alexander 1986) and cycloheximide tolerance in culture (Harrington 1981). *Leptographium procerum* was reported to cause black staining in white pine (Lackner and Alexander 1982, 1983), but this phenomenon has not been observed in recent years (Carlson 1994) and is likely caused by the presence of other fungi. Although wood staining is not believed to be caused by *L. procerum*, the wedge shaped areas of resin soaking are very similar to the colonization pattern of *Ophiostoma* spp. (Horner and Alexander 1986).

Leptographium procerum was first associated with basal cankers in *P. strobus* saplings in the late 1960's (Dochinger 1967, Houston 1969). Houston (1969) was able to

isolate *L. procerum* from cankers on eight year old *P. strobus* in the Tug Hill Plateau region of New York, but the fungus was not determined to be the primary cause of the disease. *Leptographium procerum* and *Heterobasidion annosum* (Fr.) Bref. were associated with the decline of subsoiled loblolly pine (*Pinus taeda* L.) seed orchards (Webb and Alexander 1982). Investigators were able to isolate *L. procerum* from resin soaked roots of 30% of the declining trees, but no definitive tests were performed to determine if *L. procerum* was responsible for the resin soaking or if *L. procerum* was a component of the decline. Lackner and Alexander (1982) were the first investigators to complete Koch's postulates demonstrating that *L. procerum* is pathogenic on *P. strobus* seedlings using a conidial suspension root dip inoculation. However, other workers have reported contradictory results. Wingfield (1986) and Carlson (1994) were able to infect *P. strobus* seedlings with *L. procerum*, inducing resin soaking and callus formation at the point of inoculation. Carlson (1994) found *L. procerum* colonized host tissue 2.22 ± 0.10 cm from the point of inoculation, but caused no detectable symptoms and did not kill any seedlings after 6 weeks incubation. Wingfield (1986) reported mean colonization of host tissue to be 1.62 to 2.26 cm after five months incubation using eight different isolates of *L. procerum* and was also unable to detect any symptom of disease. Despite the inability of most researchers to artificially produce the disease, PRD is a very serious disease problem of *P. strobus* in Virginia Christmas tree plantations. Based on the observation that symptomatic trees are seldom less than six years old, Carlson (1994) suggested that seedlings in the field may be inoculated in their first few years by cambium-feeding weevils and not exhibit symptoms until they near maturity as Christmas trees.

Distribution

Leptographium procerum is of cosmopolitan distribution and has been reported in the United States, Canada (Kendrick, 1962), New Zealand (Shaw and Dick 1980), and Northern and Eastern Europe (Halambek 1976; Hallaksela 1977, Wingfield and Gibbs 1991, Piou 1992, Leveux et al. 1994). Dochinger (1967) first reported infection of white pine in Indiana, Kentucky, Maryland, Ohio, Pennsylvania and West Virginia, in 3 to 15 year-old plantations. In succeeding years *L. procerum* has been isolated from trees in Florida, North Carolina, South Carolina, and Virginia, (Alexander 1980), New York (Houston 1969, Sinclair and Hudler 1980), Michigan, Minnesota, Wisconsin (Wingfield 1983), Idaho (Bertagnole et al. 1983), Alabama, Mississippi, and Tennessee (Alexander et al. 1988).

Host Range

Leptographium procerum is most commonly isolated from the genus *Pinus*. Alexander et al. (1988), documented reports of *L. procerum* infection of 12 species of pine frequently planted in North America. *Leptographium procerum* has been isolated from other conifer species including Douglas-fir (*Pseudotsuga menieszii*) (Harrington and Cobb 1983), and Fraser fir (*Abies fraseri*) (Alexander et al. 1988), and Norway spruce (*Picea abies*) (Hallakasela 1977). Although many conifers can be infected with *L. procerum*, the disease syndrome has only been described as procerum root disease in white pine and Scots pine.

Symptoms of Procerum Root Disease in White Pine

The earliest detectable symptoms of PRD are mild chlorosis, delayed bud break and

reduced shoot elongation relative to healthy cohorts (Alexander et al. 1988, Carlson 1994). These symptoms are often accompanied by localized flattening of the lower stem, where the cambium has been killed (Carlson 1994). Resinous exudate may also be visible on the lower stem, without any obvious mechanical injury. Fresh resin appears as clear droplets or rivulets and accumulated exudates are white/gray in color cascading down the lower stem from the point of origin. When the bark beneath the exudate is removed, variable brown discoloration of the cambium is observed (Lackner and Alexander 1982, Carlson 1994). Later symptoms include: marked chlorosis, very negative water potentials, reduced needle length and diameter, eventually leading to wilting and uniform reddening of the foliage (Alexander 1980, Alexander et al. 1988, Carlson 1994). When the lower stem is examined in cross section in 6 to 10 year-old plantation-grown white pine, wedge shaped zones of resin permeated wood extending from the outer xylem to the pith are observed (Carlson 1994). Evidence of bark beetle and weevil activity may be present on the lower stem of diseased trees (Alexander et al. 1988).

Insect Vectors

Early studies noted an association between symptomatic white pines and the presence of two species of cambium feeding weevils: *Hylobius pales* and *Pissodes nemorensis*, and bark beetles of the genera: *Pityokteines*, *Pityophthorus*, and *Pityogenes* (Lackner and Alexander 1983). Many species of bark beetles have been shown to vector phytopathogenic fungi, aided by specialized spore trapping/protecting structural adaptations (Francke-Grossman 1967, Whitney 1982). However, the ability of weevils to transport fungi and

successfully inoculate trees was relatively unknown. Emergence holes and breaks in the cambium caused by larval feeding were found to be sites of *L. procerum* conidial production (Lackner and Alexander 1984). To determine if bark beetles and/or weevils were contaminated with *L. procerum* in the field and potential vectors of PRD, Lewis (1985), collected insects from 10 Christmas tree plantations using fresh pine bolt traps. The authors were able to isolate *L. procerum* from *Hylobius pales* and *Pissodes nemorensis* and four genera of bark beetles (*Hylastes*, *Orthotomicus*, *Pityogenes*, and *Xyleborus*) attracted to the pine bolt traps. *Leptographium procerum* was isolated from 64% of the weevils and 0.8% of the bark beetles using selective media. Later studies demonstrated that *H. pales* and *P. nemorensis* artificially contaminated with *L. procerum* could transmit the fungus to healthy white pine seedlings while feeding on cambial tissue (Nevill and Alexander 1992a). Under field conditions *H. pales* and *P. nemorensis* are thought to become contaminated with the sticky conidia of *L. procerum* prior to emergence from a diseased brood tree, then inoculate white pines via feeding activities (Nevill and Alexander 1992b).

Physiological Impacts of Procerum Root Disease

Impregnation of sapwood with resin reduces its ability to conduct water. Horner (1985) found that resin-filled sapwood conducted water significantly less than clear sapwood. It seems likely that reduced sapwood conductivity could lead to leaf desiccation and foliar symptoms that are similar to severe water stress. Horner (1985) also found that sapwood moisture content of PRD symptomatic trees (measured at regular intervals from 15 to 90 cm along the stem) was significantly lower than that of apparently healthy neighbors. It is

difficult to determine whether the reduction of sapwood moisture content was due to the displacement of moisture by resinous occlusions or due to aspiration of otherwise functional tracheids "upstream" from irreversible basal occlusions. The reduced moisture content may simply be an expression of the degree of moisture stress present in PRD infected trees, but it may also serve to reduce xylem conductivity (Waring and Running 1978).

To measure the degree of disease severity Carlson (1994), placed basal stem segments in fast green dye and allowed them to absorb the dye for several hours. The segments were cross sectioned with a band saw to reveal green stained conducting areas and unstained occluded areas. A disease severity scale was developed using the percent occluded basal area and compared to several physiological variables. Pre-dawn water potential, daily change in pre-dawn to midday water potential, stomatal conductance, transpiration and photosynthetic rate all decrease significantly with increasing disease severity (Carlson 1994).

Carlson (1994) noted several instances where physiological measures were not commensurate with disease severity. Trees with relatively low disease severity ratings exhibited pre-dawn water potentials similar to severely diseased trees. Conversely, several trees with high disease ratings were able to maintain pre-dawn water potentials similar to uninfected trees. It is possible that the disease severity scale based on percent occluded basal area does not accurately predict the potential conductivity of the sapwood. Differences in microsite soil water availability may also contribute to this discrepancy (Carlson 1994).

Measurement of Xylem Occlusion and Hydraulic Conductivity

A means of quantifying occlusion and subsequent hydraulic conductivity of sapwood

is essential for studying how the physiology of *P. strobus* is affected by PRD. Various techniques have been used to describe and quantify the extent to which xylem elements are occluded. Dye conduction through abscised stem sections has been a useful tool to determine what proportion of vessels are mechanically blocked by hyphae, resin or other physical debris (Basham 1970, Parmeter et al. 1992, Carlson 1994).

Yamaoka et al. (1990) compared the relative obstruction of sap flow by several species of bluestain fungi, *Ophiostoma* spp. using heat pulse velocity (HPV) technology. HPV instruments enable researchers to monitor sap flow in limited areas in intact trees. Small heating elements are inserted in the sapwood downstream from a thermistor. The ascent of sap proceeds past the heating element which is periodically turned on. The time lapse between the time of heating and the time of heat detection by the thermistor is measured and the rate of flow is determined. Yamaoka et al. (1990) found significant reduction of sapwood conductivity when colonized by several *Ophiostoma* spp. HPV is most useful in forest pathology studies when the areas of tracheid blockage are uniform as with *Ophiostoma* colonization or wilt diseases. This technique would likely be inaccurate if applied to PRD affected trees where wedge shaped zones of non-functional resin soaked wood lie adjacent to functional sapwood. The arbitrary placement of the HPV sensors could be in fully functional or dysfunctional resin soaked wood giving stem conductance values that are either much higher or lower than true value.

Sperry et al. (1988) measured hydraulic conductivity of excised sugar maple (*Acer saccharum*) branches 0.4 to 0.7 cm in diameter by measuring the gravity-mediated flow of perfusing solution through branch segments. Branches measuring 45 cm in length were cut

in the field and wrapped in plastic to limit water loss until they could be analyzed. No vessels were found to be longer than 15 cm, so this length was designated as the "buffer" length on either side of the 10 to 15 cm conducting segment in the middle. The buffer lengths were cut off under water to prevent the formation of emboli. Gaskets made of rubber tubing were fitted to the conducting segments. The gaskets were attached to a raised reservoir of perfusing solution (deionized water with 10 mmol oxalic acid). Gravity-induced pressure forced water to pass through the segment at a constant pressure gradient. Solution passing through the xylem vessels was collected and weighed electronically. Baker (1993) used the same technique as Sperry et al. (1988), but applied the technique to study the reduction in root hydraulic conductivity of mature Douglas-fir and grand fir *Abies grandis* (Dougl. ex D. Don) by a root disease complex. The fungi involved in the complex were: *Heterobasidion annosum*, *Armillaria ostoyae*, *Inonotus tomentosus*, *Phaeolus schweinitzii*, *Phellinus weirii* and *Leptographium wageneri*. Baker (1993) found that root segments with staining exhibited a 30% reduction in conductivity, while those with decay were reduced by over 80%.

Lo Gullo and Salleo (1991) measured hydraulic conductivity of four-year-old *Ceratonia siliqua* L. seedlings using a method very similar to Sperry et al. (1988), to estimate the amount of cavitation caused by drought stress. They described hydraulic conductivity in terms of native flow (L):

$$L=m/(dP/dl)$$

where m is the mass flow rate through a stem segment of standard length (30cm) and dP/dl is the pressure gradient. Both Sperry et al. (1988) and Lo Gullo and Salleo (1991) used

pressure gradients of 10 kPa to measure the flow, while keeping most drought-stress-induced emboli intact. Maximum flow rate was determined by flushing out emboli with pressures of 175 kPa and remeasuring L at 10 kPa. The criticism of the technique used by Sperry et al. (1988), Lo Gullo and Salleo (1991), and Baker (1993), is the use of a positive pressure gradient to measure hydraulic conductivity. While positive root pressure does occur under some circumstances most of the water passing the sapwood of trees is drawn up by negative pressure created by transpirational forces (Kramer 1983). Nobel et al. (1990) and North and Nobel (1994) used a partial vacuum to draw solution through fine roots to measure hydraulic conductivity. Instead of collecting solution pushed through a root or stem segment, these workers measured the volume of solution pulled through the segment in a capillary pipet attached to a partial vacuum. Nobel et al. (1990) defined hydraulic conductivity (L_p) as:

$$L_p = (\Delta J_v / \Delta P)(1 / A)$$

Where ΔJ_v is the volume flow rate ($\text{m}^3 \text{s}^{-1}$), ΔP is the applied pressure differential (kPa) and A is the lateral surface area (m^2). The negative pressure technique is certainly the most physiologically correct way to measure hydraulic conductivity, but care must be taken not to use pressures great enough to break the water column and cause air emboli. Nobel et al. (1990) and North and Nobel (1994) used a pressure differential of -90 kPa and it was low enough not to break the water column. For measuring hydraulic conductivity of *P. strobus* Christmas trees 10-20 cm in diameter, negative pressure would be impractical. When using negative pressure it is crucial not to allow air to be pulled into the stem segment, typically requiring the stem to be cut under water. Positive pressure may serve to fill breaks in the

water column and forcibly remove air emboli created during cutting. Since the most practical method of cutting *P. strobus* Christmas trees is using a chainsaw in the presence of air, the selection of a positive pressure measurement technique would be most useful.

Moisture Stress and Disease Development

PRD symptoms in white pine have been reported to be particularly severe on wet, poorly drained soils (Towers 1977, Shaw and Dick 1980, Sinclair and Hudler 1980, Halambek 1981, and Smith 1991). To date, no definitive studies have established whether rates of *L. procerum* infection and colonization of host tissue or PRD disease severity are dependent on soil moisture content. Excessive soil moisture can influence the development of disease by impacting the activities of the pathogen, the host and soil microorganisms (Cook and Papendick 1972). Flooding can affect fungal propagule production and dispersal. *Phytophthora* spp. are an example of this phenomenon, where saturated conditions stimulate zoospore release by the sporangia and excessive moisture aids the dispersal of the motile spores (Stolzy and Sojka 1984). Propagules of *L. procerum* have been detected in the soil adjacent to the root collar of PRD symptomatic trees (Lewis 1985). However, the role of these propagules in infection was shown to be unimportant (Lewis et al. 1987). Since *L. procerum* is believed to be transmitted by insects (Nevill and Alexander 1992a) and the viability of spores in soils is limited (Lewis et al. 1987), it is unlikely that soil moisture has any effect on inoculum behavior.

The term *predisposition* has been used in plant pathology for many years. Yarwood (1959) defined the term as "an internal degree of susceptibility resulting from external

causes". Soil moisture extremes can predispose plants to infection, colonization and disease development (Stolzy and Sojka 1984). Towers and Stambaugh (1968) demonstrated that 10 year old, plantation grown loblolly pine roots artificially inoculated *Heterobasidion annosum* were colonized significantly faster under droughty conditions than roots grown under non-drought conditions. The authors created droughty soil conditions in the field by digging trenches around the trees and sealing the soil surface up to the tree base with plastic sheeting. Dry soil conditions were associated with reduced resistance to *H. annosum* infection in both seedlings and mature trees. The authors concluded that loblolly pine seedlings and established trees are predisposed to *H. annosum* root rot disease development by moisture stress. Other examples of tree species predisposed to pathogens by water deficits include: *Endothia gyrosa* (Schw.) canker expansion on pin oaks (*Quercus palustris* Muenchh.) (Appel and Stipes 1984) and *Botryosphaeria dothidea* (Moug. ex Fr.) invasion of peach (*Prunus persica* L. Batsch.) bark (Pusey 1989).

Excess moisture can also predispose trees to invasion by pathogens. Saturating conditions can result in a "physiological drought" where water uptake is reduced by anoxic conditions in the roots (Wargo and Harrington 1991). Under these conditions, photosynthetic activity and protein synthesis rates decrease (Kramer 1983) leading to decreased production of enzymes and metabolites that may be essential for host defense mechanisms and disease resistance (Boyer 1995). Paine and Stephen (1987) demonstrated that the inducible defense system of loblolly pine can be diminished in trees growing on waterlogged sites. *Ceratocystis minor*, a fungal associate of the southern pine beetle, was inoculated in trees growing on well-drained and wet, saturated soils. Trees in the well-drained sites reacted to the inoculation

with a greater hypersensitive response than trees on saturated sites, which limited fungal colonization. Klepzig et al. (1992) found that the growth of *L. procerum* along with *L. wagneri* and *L. terrebrantis* in red pine (*P. resinosa* L.) were reduced by phenolics and monoterpene vapors produced by the seedling. They suggested that stresses on red pine, such as excessive shading, increase susceptibility to fungal infection and colonization by reducing the concentration of defensive compounds. Goheen et al. (1978) studied the effects of soil moisture on disease development in ponderosa pine seedlings (*P. ponderosa*) surface inoculated with *L. wagneri*. Inoculations were performed by taping a 3 cm colonized wooden block to a root. High soil moisture was found to increase infectivity, but had little effect on the extent of colonization. Successful infection of ponderosa pine with *L. wagneri* in moist soil may be favored by decreased reduction-oxidization potential or possibly increased levels of soluble manganese (Goheen et al. 1978). Wilks et al. (1985) showed that *Ceratocystis wagneri* (anamorph: *Leptographium wagneri*) disease centers in ponderosa pine expanded faster under high soil moisture conditions. Not only did the fungus grow faster through the saturated soil, but infection rates of ponderosa pines increased. Wilks et al. (1985) proposed a hazard rating system for stand establishment of ponderosa pine that was based on soil moisture.

Procerum root disease is a disease where the physiological symptoms are indistinguishable from water deficit stress. Any circumstance that further limits water available for translocation to the foliage would likely heighten the degree of disease expression. Even if the infectivity of *L. procerum* or the rate of colonization in *P. strobus* is not directly affected by excess moisture, saturated conditions lead to root oxygen stress,

decreased conductivity of roots and water stress, heightening symptoms in a tree that already has a partially damaged vascular system.

Soil moisture can affect not only the pathogen and host, but can also influence the behavior of the insect vectors of disease. It is possible that soil moisture conditions may have made *P. strobus* Christmas trees more attractive to weevils and subject to more inoculation opportunities. Lavalley et al. (1994) conducted an experiment to determine if watering regime of *P. strobus* could affect feeding preferences of the white pine weevil *Pissodes strobi* (Peck). They used five and six year old potted trees in the greenhouse and exposed them to a wet (non-stressed) and dry (stressed) treatment. The workers found that *P. strobi* preferred trees in the non-stressed treatment. These trees were given 300 ml of water per day but it was unclear whether the trees were watered optimally, as indicated by the term non-stressed, or excessively, since no physiologic measures were recorded for the trees. This study did establish that *P. strobi*, a close relative to *P. nemorensis*, which can vector *L. procerum*, can differentiate between, and preferentially feed on, white pines in different watering regimes.

Effects of Weed Control on Soil Moisture.

Christmas tree plantations typically require vigorous control of both woody and herbaceous weeds. Unchecked competition from weed species for light, nutrient, and water resources may be detrimental to the success of seedlings (Kreh and Zedaker, 1989). As trees grow taller, competition for light becomes less important. Weeds are still suppressed to keep rows clear for equipment, to reduce interference with lower limbs and for aesthetic reasons. The influence of weeds and weed suppression on soil water and nutrient availability in the

immediate vicinity of 6 to 10 year-old plantation-grown Christmas trees is relatively undocumented. It would be useful to learn the impact of herbicide application in plantations, since it could be a useful tool to manage soil moisture and potentially manage diseases dependent on specific soil moisture conditions. When herbicides are used in hardwood to pine conversion programs to eliminate non-pine species, soil moisture increases significantly due to decreased transpiration losses (Sterret and Adams 1977). Sterret and Adams (1977) found that white pine seedlings grown in herbicide-converted hardwood stands were 16 to 18 cm taller than seedlings planted in hardwood stands. The reduced growth is explained by heavy competition for light and water. In plantations aged 6 to 10 years, the trees have only mild competition with weeds for light, while relative intensity of competition for water is unknown. In loblolly pine plantations in the Coastal Plain and Piedmont regions of the eastern United States, herbaceous and woody weed control results in increased height growth of trees aged 1 to 5 years (Nelson et al. 1981, Carter et al. 1984). Nelson et al. (1981) demonstrated that weeds compete directly with loblolly pine seedlings for water. Seedlings were established with and without herbaceous weed control (Velpar 0.45 kg ai/ac & 0.91 kg ai/ac). Three months later, after a 5 cm rain episode, moisture stress (PDWP) was monitored for 12 days using a pressure bomb. Seedlings established with weed control had water potentials of 0.4 to 0.5 MPa and seedlings without weed control 0.7 to 1.5 MPa. Carter et al. (1984) found similar results on 5 year old loblolly pines, though the moisture stress with no weed control was rarely over 1.0 MPa. These studies suggest that more soil moisture and or nutrient resources are available to tree crops, as evidenced by increased growth response, when herbicide is applied to competing vegetation. However, few quantitative studies of the

impact on soil moisture have been made.

Site Factors Influencing White Pine Productivity

Productivity of tree species is often closely tied to slope, aspect and specific soil factors such as drainage class, texture, available nutrients and depth of soil. Planting trees on sites appropriate for the species is essential for good health and productivity. Trees growing under optimal conditions have more effective defense systems and higher concentrations of defense compounds (Cook and Papendick 1972, Schoeneweiss 1975, Klepzig et al. 1992, and Boyer 1995). In order to study the impact of site factors on PRD development in *P. strobus* it is important to review how microsite factors contribute to *P. strobus* health. Brown and Stires (1984), developed models to predict site index of white pine in southeast Ohio from abiotic site factors. They identified aspect, distance from ridge (position), total soil depth and depth of the A horizon to be useful for predicting white pine productivity on different sites. The authors observed that site index value increased in direct proportion to total depth of soil, which varied from 30 to 90 cm. Similar results were observed as the depth of the A horizon increased from 5 to 20 cm. Greatest productivity was found on northeast aspects followed by southeast and southwest slopes. Site index values increased from ridge to upper slope, mid slope, lower slope, and was greatest in the bottom. Sites at the lower slopes are thought to be more productive due to increased soil depth, better soil development and greater moisture availability. Productivity of white pine generally decreases as the pH of the surface mineral layers increases (Stratton and Struchtemeyer 1968). Williams et al. (1990) documented that white pine was most productive on soils with sandy textures and developed

more slowly in clayey soils in southern Ontario. White pine on clay soils took on average 6.1 years to reach breast height, while trees on loamy soils took 5.0 years and trees on sandy soils took 4.9 years. There was no significant difference in years to breast height across 3 drainage classes: moist to very moist regime 5.0 years, fresh to moderately moist regime 5.3 years, and fresh to dry regime 5.3 years. In Maine, Stratton and Struchtemeyer (1968) found highest white pine productivity on: moderately well drained, well drained and excessively drained soils. Inhibited growth was reported on sites that were somewhat poorly drained and poorly drained.

Chapter 2

Influence of Site Factors on the Physiology of Eastern White Pine (*Pinus strobus* L.) with Procerum Root Disease in Christmas Tree Plantations.

INTRODUCTION

Procerum root disease (PRD) is a serious problem in Virginia in eastern white pine (*Pinus strobus* L.) Christmas tree plantations (Alexander 1989). *Leptographium procerum* (Kendr.) Wingf., the causal agent of PRD has been routinely isolated from pales weevils *Hylobius pales* (Herbst.) captured in plantations and is believed to be vectored by these insects (Nevill and Alexander 1992a). Extensive monocultures of *P. strobus* in Christmas tree production serve to intensify insect and disease problems. After trees are harvested in late fall, a multitude of stumps from both healthy and diseased trees remain and serve as brood trees for the pales weevil (Anderson 1980, Carlson 1994). The weevils are thought to become contaminated with the sticky conidia of *L. procerum* in the diseased stumps and disseminate the fungus to other seedlings and trees (Nevill and Alexander 1992b).

Many investigators have associated PRD with trees growing on poorly drained soils in low lying areas (Towers 1977, Shaw and Dick 1980, Sinclair and Hudler 1980, Halambek 1981, and Smith 1991). It is unclear whether this relationship is real or merely anecdotal. It is also unclear whether excessive soil moisture elicits more rapid disease development or is related to the feeding and reproductive behaviors of the insect vectors (Nevill 1990).

The objective of this study was to assess the role of soil-site factors (soil moisture,

slope, aspect, position, texture, bulk density, porosity and pH) on the physiology of healthy and diseased *P. strobus* in Christmas tree plantations. Leaf conductance was used as a measure of symptom expression and disease severity was indexed by three vascular variables: hydraulic conductivity of the basal stem, percent basal occlusion and sapwood moisture content. Soil moisture was the primary soil-site variable of interest, since it was a dynamic measure that could be recorded periodically in conjunction with leaf conductance and other foliar variables. Plots were selected in a diversity of drainage classes to obtain a soil moisture gradient from moderately dry to very wet. It has been suggested that herbicide application may make more water available to trees than does mowing (Kreh and Zedaker 1989; Torbert personal communication, Department of Forestry, VPI & SU), therefore, two weed control techniques were used in each plot to exaggerate intra-plot soil moisture differences.

MATERIALS AND METHODS

Selection of Study Sites

Two *P. strobus* Christmas tree plantations in Floyd County, Virginia were used for the field investigation. Selection of study plots at each site was based on three factors; presence of an adequate number of diseased trees for study, an adequate number of healthy trees in close proximity to diseased ones, and plots that exhibited substantially different soil moisture regimes. At each site, plots were selected to represent a gradient from moist to dry soil conditions and to encompass a variety of topographic features (slope, aspect and position). Four plots approximately 50m in diameter were established at each plantation. Individual plot size and shape varied depending on the distribution of the sample trees within

the plot.

Plantation 1 was under active cultivation and consisted of 8 hectares of *P. strobus* and *P. sylvestris* L. Christmas trees. This plantation included plots that exhibited relatively low soil moisture contents. Plantation 2 consisted of 20+ hectares of *P. strobus* Christmas trees and has not been actively producing trees for 3-4 years. This site was selected due to an abundance of diseased trees and the very wet conditions found in areas of the plantation. Plot characteristics are summarized in Table 2.1.

Selection of Sample Material

Six healthy and 12 visually symptomatic trees were selected in each of the eight plots. Every effort was made to choose 12 trees grading from barely to noticeably symptomatic. In June of 1994, trees were selected with delayed bud break or retarded shoot elongation that otherwise appeared healthy. Trees exhibiting more severe symptoms including: marked chlorosis, flattened areas on the lower stem, basal resinosis, and reduced shoot elongation for several seasons were also chosen. Visually healthy control trees were situated in the same plot interspersed with the diseased trees.

Cultural Treatments.

To evaluate the effect of mowing versus herbicide application, competing vegetation in the two meters surrounding one-half of the diseased and healthy trees in each plot was mowed. The remaining half of the trees received an application of Roundup® (isopropylamine salt of glyphosate 41% a.i.)(Monsanto, Inc.) at 1.875 % a.i. until foliage was lightly wetted

Table 2.1. Topographic characteristics of plantations and plots used in study. Both plantations were located in Floyd Co., VA.

	% Slope	Aspect	Position	Relative Moisture Class ¹
Plantation 1				
Plot 1	5 - 12 %	315 ⁰ - 35 ⁰	summit/bowl	moist
Plot 2	7 - 14 %	320 ⁰ - 10 ⁰	backslope/footslope	moderately moist
Plot 3	14 - 20 %	265 ⁰ - 320 ⁰	backslope/shoulder	moderately dry
Plot 4	12 - 19 %	250 ⁰ - 320 ⁰	backslope	moderately dry
Plantation 2				
Plot 5	0 - 9%	315 ⁰ - 20 ⁰	summit/bowl	very wet
Plot 6	1 - 17 %	0 ⁰ - 15 ⁰	backslope/footslope	very moist
Plot 7	14 - 18 %	295 ⁰ - 320 ⁰	backslope/shoulder	moist
Plot 8	0 - 9 %	245 ⁰ - 290 ⁰	backslope	very wet

¹ estimated relative moisture class; later quantified with TDR

on July 1, 1994.

Gas Exchange.

Leaf conductance, transpiration and diffusive resistance were measured with a Li-Cor 1600 steady state porometer fitted with a 4 cm² closed system cuvette (Li-Cor Inc., Lincoln, NE)(Bingham and Coyne 1977, Schultze et al. 1982). Measurements were made every 7 to 10 days from July 15 to October 15, 1994. Measurements were made using one fascicle excised from each tree. Leaf conductance values were later corrected using the total surface area of each fascicle. Surface area was computed with the following equation:

$$SA = 3.14159(d)(l) + (n)(d)(l)$$

Where d = fascicle diameter, l = needle length, and n = the number of needles in a fascicle (Ginn et al. 1991). Fascicle diameter was measured in the field with digital calipers to the nearest 0.05 mm.

Soil-Site Measures.

Volumetric water content of the soil immediately adjacent to each tree was measured at depths of 10 and 20 cm with Time Domain Reflectometry (TDR)(Model 6050X1 Trase System, Soilmoisture Equip. Corp., Santa Barbara, CA)(Top et al. 1984). Paired probes were installed at each depth on the north side of the tree, within the dripline. Measurements were recorded the same day as gas exchange measures.

Basic soil characteristics were analyzed at the end of the study. Slope, position, and

aspect were recorded adjacent to each tree. Soil cores were collected at three randomly selected locations from the A and B horizons in each plot. Cores were used to measure bulk density, hydraulic conductivity and porosity (Brady 1984, Burger 1994). A push-tube was used to collect samples from the A and B horizons from 10 randomly selected locations in each plot. The samples were oven dried, and composited into one sample for each plot. These samples were used to determine pH and soil texture. Texture was determined at using the hydrometer method (Bouyoucos 1962). Mean values for all soil-site variables documented are presented in Table 2.2.

Vascular Conductivity Measures.

At the end of the soil moisture and physiology studies, in late October, the trees were systematically harvested for hydraulic conductivity analysis. Nine trees per day were harvested for 16 consecutive days. Trees were cut as close to the ground as possible and returned to the laboratory. The trees were re-cut to remove any soil or sap accumulation on the cut surface. A 5 cm long segment was removed, debarked and soaked in clean tap water until hydraulic conductivity measures were made.

To measure xylem hydraulic conductivity (L_p) an apparatus was developed using the basic principles of Sperry et al. (1988), but modified to accommodate debarked stem segments of up to 20 cm in diameter. Constant pressure to force water through the excised segment was created by placing a 20 l container up a pole to a height of 4 m with a rope and pulley. The water level could be maintained via a hose attached to the raised reservoir. This configuration was able to create a pressure differential of 40 kPa. The water head was fitted

Table 2.2. Mean soil characteristics for plantations and plots used in study. Both plantations were located in Floyd Co., VA.

	Plantation 1								Plantation 2							
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
Bulk density A horizon (g/cm ³)	1.35	1.43	1.44	1.39	1.11	1.28	1.14	1.27	1.35	1.43	1.44	1.39	1.11	1.28	1.14	1.27
Bulk density B horizon (g/cm ³)	1.39	1.62	1.43	1.63	1.30	1.53	1.38	1.26	1.39	1.62	1.43	1.63	1.30	1.53	1.38	1.26
Capillary porosity A horizon (%)	37.46	45.01	40.22	43.92	56.46	29.83	47.18	46.02	37.46	45.01	40.22	43.92	56.46	29.83	47.18	46.02
Capillary porosity B horizon (%)	44.03	37.00	45.36	36.63	49.15	35.97	43.37	51.93	44.03	37.00	45.36	36.63	49.15	35.97	43.37	51.93
Clay A horizon (%)	18.00	10.00	10.00	10.00	14.00	14.00	8.00	20.00	18.00	10.00	10.00	10.00	14.00	14.00	8.00	20.00
Clay B horizon (%)	40.00	20.00	18.00	22.00	22.00	24.00	22.00	40.00	40.00	20.00	18.00	22.00	22.00	24.00	22.00	40.00
Non-capillary porosity A horizon (%)	11.53	1.06	5.51	3.75	1.48	22.05	9.62	5.94	11.53	1.06	5.51	3.75	1.48	22.05	9.62	5.94
Non-capillary porosity B horizon (%)	3.66	1.85	0.81	1.68	1.89	6.43	4.72	0.66	3.66	1.85	0.81	1.68	1.89	6.43	4.72	0.66
Sand A horizon (%)	26.00	38.00	36.00	40.00	48.00	34.00	60.00	46.00	26.00	38.00	36.00	40.00	48.00	34.00	60.00	46.00
Sand B horizon (%)	28.00	20.00	28.00	26.00	30.00	4.00	52.00	36.00	28.00	20.00	28.00	26.00	30.00	4.00	52.00	36.00
Silt A horizon (%)	56.00	52.00	54.00	50.00	38.00	52.00	32.00	34.00	56.00	52.00	54.00	50.00	38.00	52.00	32.00	34.00
Silt B horizon (%)	32.00	60.00	54.00	52.00	48.00	72.00	26.00	24.00	32.00	60.00	54.00	52.00	48.00	72.00	26.00	24.00
Soil hydraulic conductivity A horizon (cm/hr)	1.88	1.30	0.80	0.67	0.83	8.98	7.97	0.90	1.88	1.30	0.80	0.67	0.83	8.98	7.97	0.90
Soil hydraulic conductivity B horizon (cm/hr)	0.15	0.27	0.57	0.33	1.02	5.12	4.70	0.06	0.15	0.27	0.57	0.33	1.02	5.12	4.70	0.06
Total porosity A horizon (%)	48.98	46.06	45.73	47.67	57.95	51.88	56.80	51.96	48.98	46.06	45.73	47.67	57.95	51.88	56.80	51.96
Total porosity B horizon (%)	47.69	38.85	46.17	38.31	51.05	42.40	48.09	52.29	47.69	38.85	46.17	38.31	51.05	42.40	48.09	52.29
pH A horizon	5.62	5.08	5.03	5.08	5.44	5.95	5.67	5.82	5.62	5.08	5.03	5.08	5.44	5.95	5.67	5.82
pH B horizon	5.39	5.06	5.06	5.90	5.65	5.95	5.61	5.82	5.39	5.06	5.06	5.90	5.65	5.95	5.61	5.82

to a pressurizing unit which delivered pressurized water to the stem segments. The pressurizing unit was constructed of 10 cm PVC pipe. The hose provided the water and pressure, while the faucet on the other side was used to bleed out air and drain the system after a measurement. Different sized adapters could be fitted to pressurizing units via screw threads to accommodate various tree diameters.

To make measurements, lateral stem segments were fitted to the pressurizing unit with a rubber gasket and the unit was filled with water from the reservoir. The pressuring unit was hung upside-down to prevent air bubbles from collecting against the stem segments. Pressure from the raised reservoir was applied, forcing H₂O through the stem segment at constant pressure. After a period of stabilization, the volume of H₂O expressed in a given period was measured. L_p (cm s⁻¹ kPa⁻¹) was then expressed in the following terms:

$$L_p = (\Delta J_v / \Delta P)(1/ A)$$

Where ΔJ_v (cm³ s⁻¹) is volume flow rate, ΔP (kPa) is applied pressure difference and A (cm²) is the lateral surface area (Nobel et al. 1990).

Sapwood Moisture Content Measures.

Before L_p was measured, the debarked stem segments were weighed. After conductivity analysis, each segment was oven dried and reweighed to calculate initial percent moisture content (Panshin and de Zeeuw 1980).

Basal Occlusion Measures

Once the 5 cm segments were removed for Lp analysis, the remaining bolt was soaked in 0.5 g/l Fast Green FCF solution (U.S. Biochemical Corporation, Cleveland, OH) for 24 hours. The following day a thin disk was cut from each bolt with a bandsaw revealing a pattern of green staining on the lateral surface. Unobstructed vessels stained blue/green, while resin soaked and other dysfunctional vessels remained unstained (Basham 1970, Parmeter et al. 1992, Carlson 1994). The segments were scanned with a high resolution color scanner using a Macintosh Power PC and Adobe Photoshop (Adobe Systems, Inc., Mountain View, CA). The unstained area was determined from the scan and percent basal occlusion was calculated.

Data Analysis

All data were analyzed using SAS software on the Virginia Tech mainframe computer or SAS for Windows on a personal computer (SAS Institute, Cary, NC). All percent scale data were arcsin transformed ($\arcsin(\% \text{ variable}^{0.5})$). Leaf conductance was normalized by dividing leaf conductance values by the median leaf conductance for each sampling date to remove any effects of date. Preliminary analyses of relationships between measures of disease severity, and soil and environmental parameters included stepwise regression and correlation analysis. Mean separation analysis, when appropriate, was accomplished using the general linear model (GLM) procedure with Tukeys HSD test ($\alpha = 0.05$). A one way analysis of variance was first applied to determine the influence of health class (diseased or healthy) on physiological variables. All analyses were first run separately for each measurement date and

then re-run using leaf conductance normalized by sampling date. For the variables L_p , basal occlusion and sapwood moisture content, only trees that survived to the end of the study were measured and included in the analysis. Regression analysis was used to determine the relationship between leaf conductance and measures of disease severity (L_p , basal occlusion, sapwood moisture content). T-tests were performed to determine if there was any difference in soil moisture at 10 cm and 20 cm, and leaf conductance between the mowing and herbicide treatments. A series of regression analyses were performed using leaf conductance, L_p , basal occlusion and sapwood moisture content as the dependent variables to evaluate which site factors might be related to disease severity and symptom expression. Preliminary analysis consisted of running stepwise regression using the forward, backward, and straight regression options on the following independent variables for both the A and B horizons: soil moisture 10cm (%), soil moisture 20cm (%), azimuth, slope, silt (%), sand (%), clay (%), capillary porosity, non-capillary porosity, total porosity, bulk density, pH, and soil hydraulic conductivity. Variables that were significant in at least two of the three stepwise regression options used were considered further using the general linear models procedure.

RESULTS and DISCUSSION

Influence of disease class. Procerum root disease has a profound effect on foliar and vascular physiology of *P. strobus*. Daily mean stomatal conductance differed significantly between healthy and diseased trees on each sampling date (Figure 2.1). Stem Lp and sapwood moisture content (%) were significantly higher in healthy trees, while mean basal occlusion was greatest for the diseased trees (Table 2.3). Infection and colonization of host sapwood by *L. procerum* induces the host to fill affected regions with resins to limit fungal colonization. Under most circumstances this would be an effective strategy to slow or repel invading insects or fungal pathogens, however, *L. procerum* has the unique ability to survive and colonize resin-soaked woody tissue (Horner 1985). Instead of limiting fungal colonization, the host is systematically reducing its ability to translocate water. Diseased trees show greatly reduced stomatal conductance resulting from reduced capacity to conduct water, caused by resin infiltration of tracheids. As water conducting vessels are filled with resin and rendered non-conductive, moisture content of the sapwood is reduced (Table 2.3).

Relationship of leaf conductance to stem hydraulic properties. One of the major difficulties in quantifying the effect of procerum root disease on *P. strobus* over time was the lack of methodology to measure the severity of disease non-destructively. Disease severity ratings were previously based on percent sapwood occlusion based on dye infiltration, effectively showing which vessels were blocked with resin in a two dimensional segment of the stem (Carlson 1994). Other measures of severity used in my thesis studies included

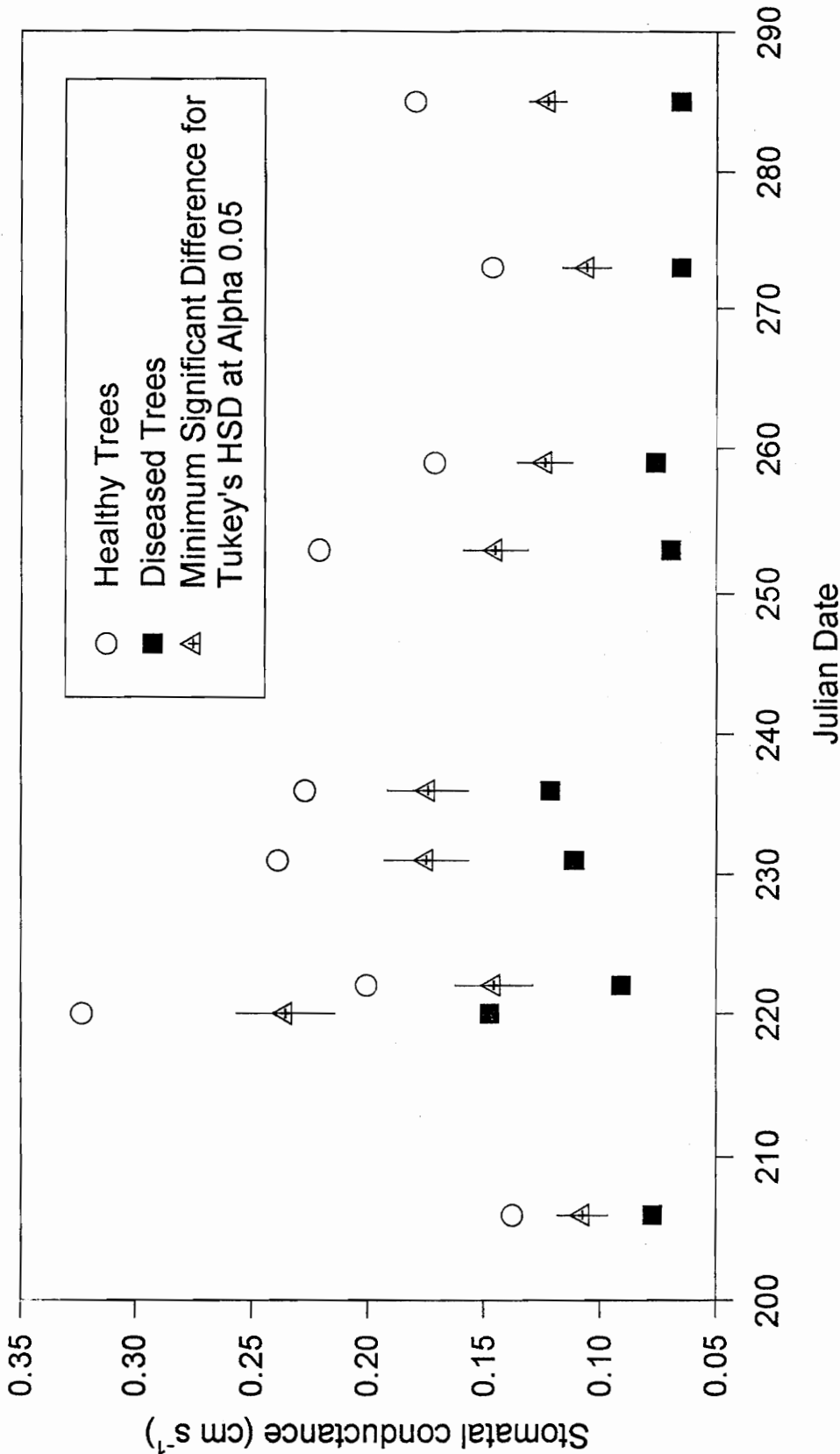


Figure 2.1. Stomatal conductance of healthy and diseased eastern white pine over the duration of the field study. Differences in leaf conductance were significant on all dates using Tukey's HSD test ($\alpha = 0.05$).

Table 2.3. Influence of disease class on stem hydraulic properties.

Disease Class	Lp (cm s ⁻¹ kPa ⁻¹)	Basal occlusion (%)	Sapwood moisture (%)
Healthy	3.24 a ¹	4.51 b	206.30 a
Diseased	0.69 b	43.60 a	81.47 b

¹ Column means with the same letter are not significantly different using Tukey's HSD test ($\alpha = 0.05$).

hydraulic conductivity and sapwood moisture content of the basal stem, which are also destructive measures. As evidenced by Figure 2.1, leaf conductance can be effectively used to determine which trees are diseased on any given day, but the values overlap among sampling days. Since there are no non-destructive measures of disease severity we used leaf conductance to monitor disease severity on living trees during the growing season. To use leaf conductance in this manner it was essential to understand its relation to the vascular disease severity variables. Lower leaf conductance was found in conjunction with lower stem L_p , higher basal occlusion, and lower sapwood moisture content (Tables 2.4, 2.5), indicating that leaf conductance is a symptom of increasing disease severity. Basal occlusion and sapwood moisture content explained more of the variation than L_p in leaf conductance at each sampling date and at all dates using leaf conductance values normalized by sample date using linear regression (Figure 2.2 a, b, c). This is likely due to the high degree of variability in the L_p measurements. There is much less error inherent in the measurement of occlusion and sapwood moisture. Measurement of L_p is difficult, time consuming, destructive and explained less of the variation in leaf conductance than basal occlusion, but did quantify the effect of occlusion on stem physiology in *P. strobus*. Heat pulse velocity measures would have been a more accurate way to measure sapflow in a small section of the xylem at a specific point in time, but the irregular wedge patterns of resin infiltration are not distributed evenly across the lateral surface of the stem, requiring the use of a total stem conductivity measure.

Day 273, near the end of the experiment, was a favorable measurement period for high conductance, and characterized the statistical trends of all the sampling dates using non-

Table 2.4. Comparative affects of Lp, percent basal occlusion, and percent sapwood moisture content of eastern white pine using linear regression analysis.

Date	leaf conductance vs Lp			leaf conductance vs basal occlusion			leaf conductance vs sapwood moist.					
	R ²	intercept	slope	p > F	R ²	intercept	slope	p > F	R ²	intercept	slope	p > F
206	0.33	0.0629	0.0221	0.0001	0.31	0.1285	-0.0010	0.0001	0.41	0.0311	0.0005	0.0001
219	0.31	0.1441	0.0535	0.0001	0.48	0.3209	-0.0032	0.0001	0.39	0.0528	0.0013	0.0001
221	0.47	0.0695	0.0400	0.0001	0.48	0.1962	-0.0020	0.0001	0.50	0.0216	0.0008	0.0001
230	0.51	0.0966	0.0429	0.0001	0.61	0.2384	-0.0024	0.0001	0.57	0.0367	0.0010	0.0001
235	0.43	0.1032	0.0394	0.0001	0.51	0.2348	-0.0022	0.0001	0.44	0.0545	0.0008	0.0001
252	0.58	0.0686	0.0452	0.0001	0.58	0.2128	-0.0023	0.0001	0.66	0.0034	0.0010	0.0001
259	0.49	0.057	0.0335	0.0001	0.52	0.1633	-0.0018	0.0001	0.63	0.0095	0.0008	0.0001
273	0.52	0.0541	0.0271	0.0001	0.57	0.1421	-0.0015	0.0001	0.60	0.0168	0.0006	0.0001
284	0.36	0.0575	0.0166	0.0001	0.48	0.1155	-0.0011	0.0001	0.46	0.0323	0.0004	0.0001
all dates ¹	0.42	0.1902	0.4211	0.0001	0.49	63.29	-35.16	0.0001	0.49	53.69	82.56	0.0001

¹ leaf conductance normalized by date

Table 2.5. Correlation coefficients (r) among all disease severity variables¹.

	Leaf conductance	Lp	Basal occlusion	Sapwood moisture content
Leaf conductance	1.0000	0.5161	-0.5932	0.5912
Lp	-	1.0000	-0.7001	0.8736
Basal occlusion	-	-	1.0000	-0.8690
Sapwood moisture content	-	-	-	1.0000

¹ all coefficients were highly significant (P = 0.0001)

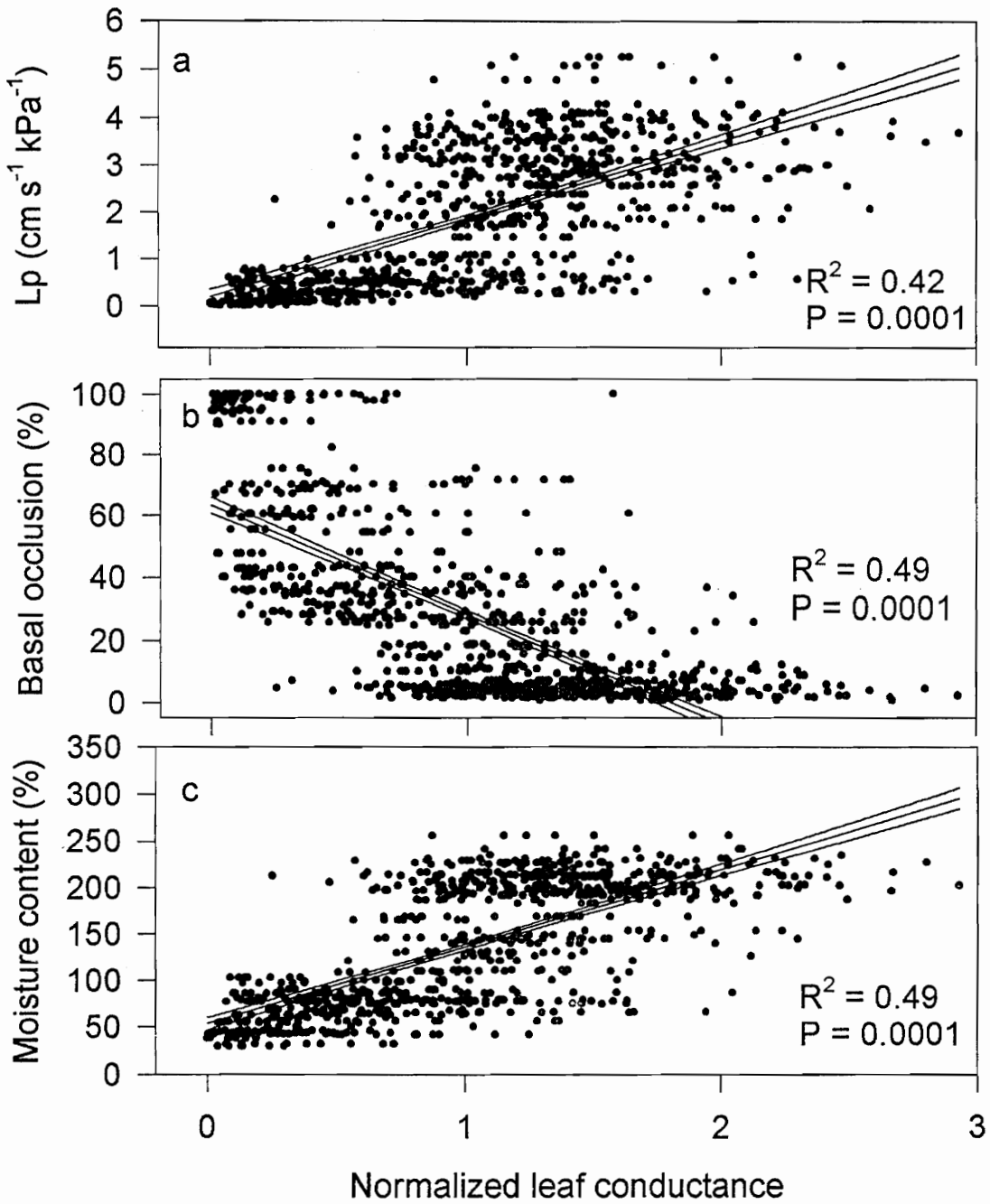


Figure 2.2. a, b, c. Effect of Lp, percent basal occlusion and sapwood moisture content on leaf conductance (\pm 95% C.I.) in *P. strobus*. Leaf conductance was normalized by sampling date.

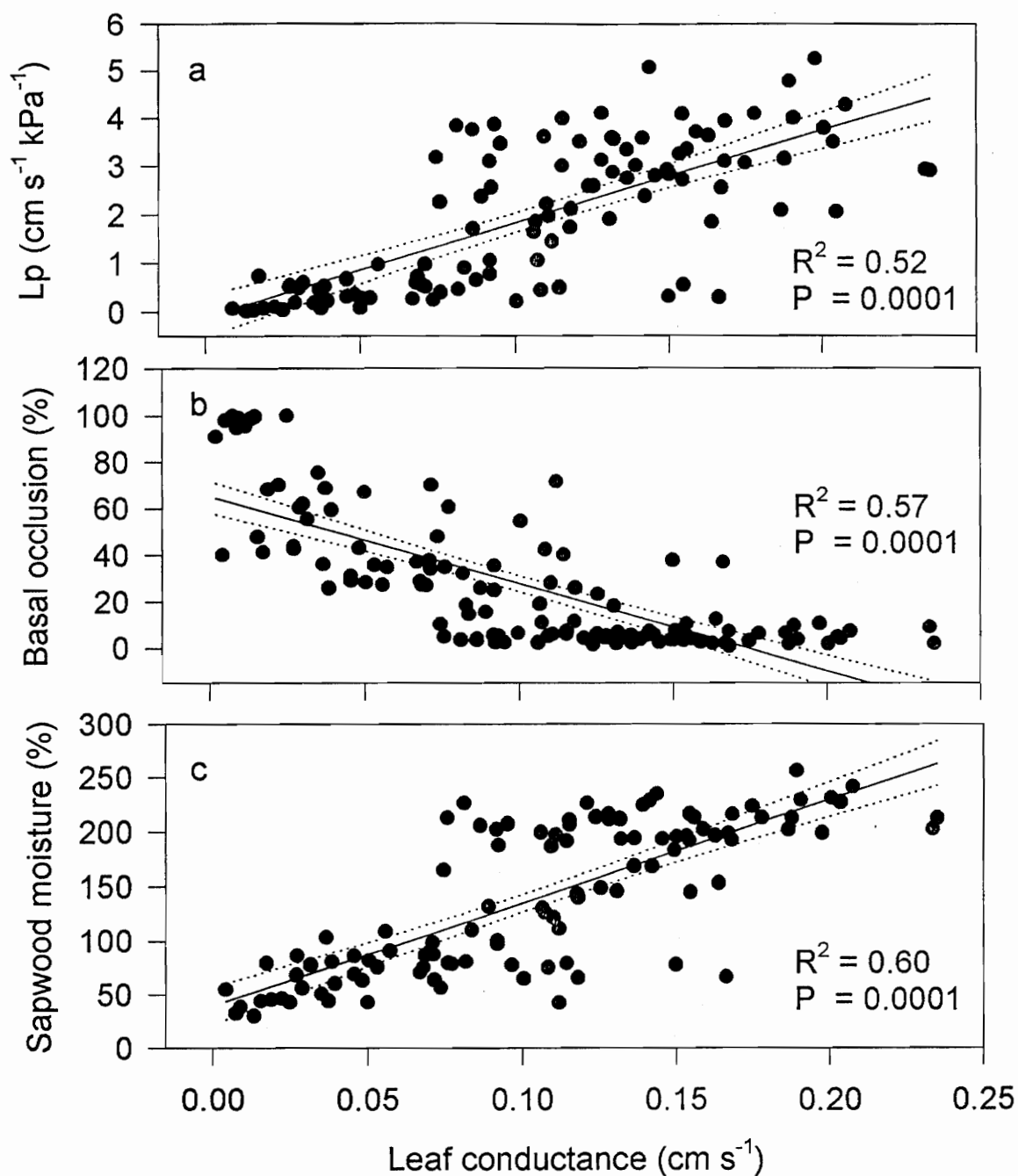


Figure 2.3. a, b, c. Effect of Lp, percent basal occlusion and sapwood moisture content on leaf conductance (\pm 95% C.I) in *P. strobus* on day 273.

normalized conductance values (Figure 2.3 a, b, c). From these results it appears that foliar disease expression is simply a matter of fluid dynamics, where water available to the leaves is reduced over time and the leaves respond accordingly.

Foliar symptoms of procerum root disease are essentially indistinguishable from those associated with water deficit stress. Fungal infection and colonization of cambium and sapwood tissue leads to the infiltration of tracheids with resin. Basal occlusion causes water stress in trees with PRD and reduced leaf conductance, stem L_p and sapwood moisture content are the physiological consequences of the primary cause. Using normalized leaf conductance to monitor disease severity allowed us to study the physiological response of diseased trees under changing soil moisture conditions. It was also important to examine the influence of site factors on disease severity variables other than basal occlusion. Carlson (1994) found that disease severity indexed by basal occlusion didn't always explain the observed foliar water status. There were trees with low disease severity exhibiting low pre-dawn ψ indicating that they were unable to fully re-hydrate from the previous day's transpirational water losses and there were trees with high disease severity that were associated with pre-dawn ψ similar to healthy trees. Carlson (1994) suggested that soil moisture variation may be responsible for this phenomenon. An alternative explanation is that occlusion at one point in a two dimensional segment of basal stem may not be indicative of hydraulic conductivity of the whole stem.

Relations among stem hydraulic variables. When initiating these studies it was difficult to select the best measure of disease severity. Resin occlusion is the primary cause

of all of the other disease severity variables, but the conductivity of sapwood has greater relevance to the water status of the foliage. Sapwood moisture content is also an excellent indicator of severity, it does not matter if tracheids are filled with resin or air emboli they are still devoid of water and non-functional. Regression analysis revealed a strong relationship between basal occlusion and stem Lp (Figure 2.4). As basal occlusion increased, making tracheids impermeable to water, stem Lp dropped markedly. When the stem segments exhibited 20 % occlusion, stem Lp was reduced over 50 % and stem Lp approached zero at basal occlusion greater than 50 %. Displacement of water from the sapwood by resin and/or air emboli reduced sapwood moisture content and impeded the flow of water, reducing stem Lp (Figure 2.5 a, b). Infiltration of tracheids with resin (increased basal occlusion) resulted in the reduction of water in the sapwood. Waring and Running (1978) found that even small reductions in sapwood moisture content can result in significant reductions in xylem Lp. Despite the inter-related or inter-dependent nature of these disease severity variables and symptoms, it was useful to analyze the effects of site factors on all of them. If an independent soil-site factor is truly affecting disease severity it should have a significant influence over all of the disease severity variables as well as the symptoms of PRD.

Impact of weed control technique on soil moisture and leaf conductance. It was surprising that no difference in soil moisture was found between the two weed control treatments at 10 cm or 20 cm (Table 2.6). Herbicide application for the control of herbaceous weeds is known to improve the water status of crop tree species (Morris et al. 1993, Mitchell et al. 1993). However, most effects of herbaceous weed competition occur in the first few

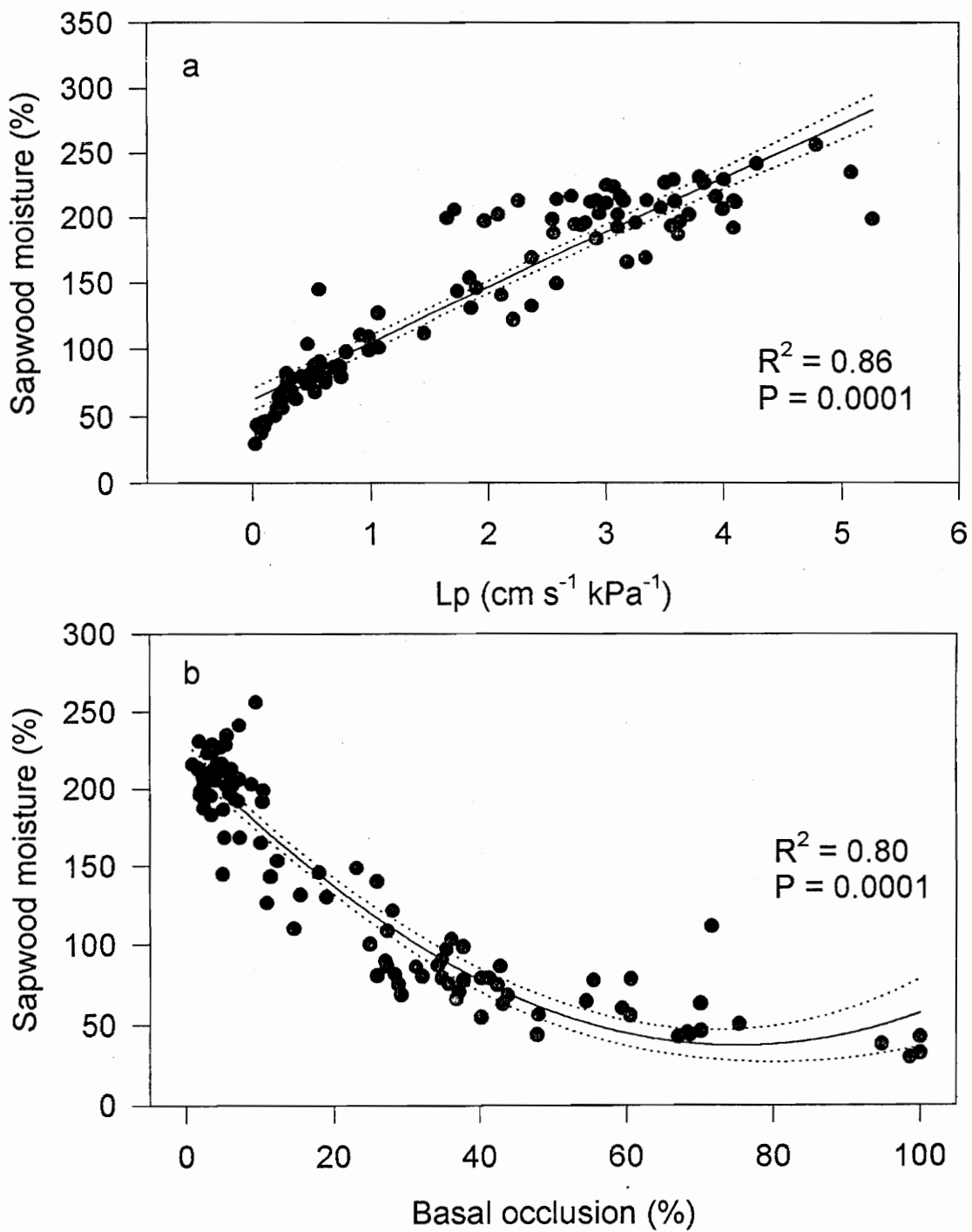


Figure 2.5. a, b. Relationship between L_p and basal occlusion (%), and sapwood moisture content ($\pm 95\%$ C.I.) in *P. strobus*.

Table 2.6. Analysis of weed control methods on soil moisture at 10 cm, 20 cm and on leaf conductance using T tests.

Source	Weed control treatment						Analysis	
	Mowing			Herbicide			F	p > F
	DF	Mean	Std. Dev.	DF	Mean	Std. Dev.		
Soil moisture 10 cm	428	23.03	7.41	430	23.09	7.76	1.54	0.6799
Soil moisture 20 cm	428	27.08	0.20	430	26.64	6.37	1.08	0.4042
Leaf conductance ¹	498	0.98	0.55	504	1.00	0.59	1.14	0.1544

¹ normalized by date

years of plantation establishment (Sands and Nambiar 1984). Likely these trees were past the stage of development where herbaceous weeds continue to influence their development. Spatial distribution of root systems and narrow radius of weed control likely influenced the observed response. No difference in leaf conductance was found between mowing and herbicide applications (Table 2.6). This demonstrates that there was no physiological effect of the weed control technique on the study trees.

Relations of Site Factors and Disease Severity. Linear regression summaries for the disease symptom and severity variables: leaf conductance, stem Lp, basal occlusion (%) and sapwood moisture content (%), and site variables are presented in Tables 2.7, 2.8, 2.9, and 2.10 respectively. Soil-site variables explained the greatest amount of variation in stem Lp (21.1 %) followed by basal occlusion (13.1 %), leaf conductance (12.7 %) and sapwood moisture content (11.6 %). Soil moisture had the greatest impact of any site variable on the suite of disease severity indicators monitored in this study. Reduced leaf conductance, stem Lp and sapwood moisture content, and increased basal occlusion were observed in conjunction with increased soil moisture at 20 cm (Figure 2.6 a, b, c, d).

Leaf conductance was significantly influenced by several soil variables: soil moisture at 20 cm and 10 cm, total porosity of soil of both the A and B horizons, bulk density of both the A and B horizons (Table 2.7). Soil moisture at 20 cm explained the greatest amount of variation in leaf conductance, but was closely followed by the other significant variables. There was significant colinearity between soil moisture at 20 cm and soil moisture at 10 cm ($r = 0.7606$, $P = 0.0001$), total porosity of the A ($r = 0.4623$, $P = 0.0001$) and B ($r = 0.4281$,

Table 2.7. Linear regression summary of independent soil-site variables affecting leaf conductance.

	Leaf conductance (cm s ⁻¹)						
	df	mean	std. dev.	intercept	slope	F value	p > F
Soil moisture 20 cm (%)	1	26.85	6.38	0.2216	-2.9083	14.61	0.0001
Soil moisture 10 cm (%)	1	23.07	7.58	0.2502	-4.6445	7.88	0.0051
Total porosity A horizon (%)	1	50.14	4.28	0.2535	-2.2111	7.55	0.0061
Total porosity B horizon (%)	1	44.81	4.85	0.2405	-2.1844	7.27	0.0071
Bulk density B horizon (g/cm ³)	1	1.50	0.27	0.0961	0.0345	7.09	0.0079
Bulk density A horizon (g/cm ³)	1	1.37	0.37	0.0579	0.0671	7.07	0.0080
pH A horizon	1	5.40	0.37	0.1079	0.0064	3.56	0.0596
Soil hyd. cond. A horizon (cm/hr)	1	2.31	3.58	0.1426	0.0017	3.18	0.0748
Non-capillary porosity B Horizon (%)	1	2.69	1.87	0.1377	0.0034	2.26	0.1333
Model	8					13.63	0.0001
							R ² = 0.127

Table 2.8. Linear regression summary of independent soil-site variables affecting L_p .

	L_p ($\text{cm}^{-1} \text{ s}^{-1} \text{ kPa}^{-1}$)						
	df	mean	std. dev.	intercept	slope	F value	p > F
Soil moisture 20 cm (%)	1	26.85	6.38	3.3321	-0.0519	34.71	0.0001
Silt A horizon (%)	1	47.50	12.27	1.2708	0.0270	31.24	0.0001
Slope (%)	1	11.81	4.81	1.5608	0.0350	6.07	0.0140
Sand B horizon (%)	1	27.02	8.63	2.8616	-0.0185	1.37	0.2426
Soil hyd. cond. A horizon (cm/hr)	1	2.31	3.58	1.9231	0.0203	0.96	0.3279
Bulk density A horizon (g/cm^3)	1	1.36	0.37	1.4779	0.0024	0.94	0.3318
Model	5					21.10	0.0001
							$R^2 = 0.211$

Table 2.9. Linear regression summary of independent soil-site variables affecting basal occlusion (%).

	Basal occlusion (%)						
	df	mean	std. dev.	intercept	slope	F value	p > F
Soil moisture 20 cm (%)	1	26.85	6.38	-9.07	1.3832	94.06	0.0001
Slope (%)	1	11.81	4.85	27.91	0.0084	7.75	0.0055
Bulk density A horizon (g/cm ³)	1	1.36	0.37	48.89	-15.7201	3.69	0.0549
Bulk density B horizon (g/cm ³)	1	1.50	0.27	61.26	-22.6305	3.42	0.0647
Capillary porosity B horizon (%)	1	42.64	6.99	28.46	-0.1438	3.29	0.0701
Silt A horizon (%)	1	47.50	8.63	43.75	-0.3277	2.10	0.1473
Non-capillary porosity A horizon (%)	1	50.15	4.28	28.57	-0.0668	1.17	0.2796
pH B Horizon	1	5.51	0.37	45.84	-3.2258	0.45	0.5049
Model	7					15.33	0.0001
							$R^2 = 0.131$

Table 2.10. Linear regression summary of independent soil-site variables affecting sapwood moisture content (%).

	Sapwood moisture content (%)						
	df	mean	std. dev.	intercept	slope	F value	p > F
Soil moisture 20 cm (%)	1	26.85	6.38	212.61	-2.7378	67.84	0.0001
Slope (%)	1	11.81	4.85	144.89	-0.4413	6.00	0.0146
Silt A horizon (%)	1	47.50	8.63	162.55	-0.4791	0.99	0.3196
Soil hyd. cond. B horizon (cm/hr)	1	1.50	1.93	141.18	-0.9317	0.52	0.4728
Bulk density A horizon (g/cm ³)	1	1.37	0.37	127.46	9.2761	0.25	0.6166
Non-capillary porosity B horizon (%)	1	2.69	1.87	145.77	-2.1580	0.16	0.6864
Capillary porosity A horizon (%)	1	42.64	6.99	133.46	0.1468	0.00	0.9777
Non-capillary porosity A horizon (%)	1	7.47	6.44	141.84	-0.2743	0.00	0.9773
Total porosity A horizon (%)	1	50.15	4.28	153.13	-0.2676	0.00	0.9643
Model	8					10.72	0.0001

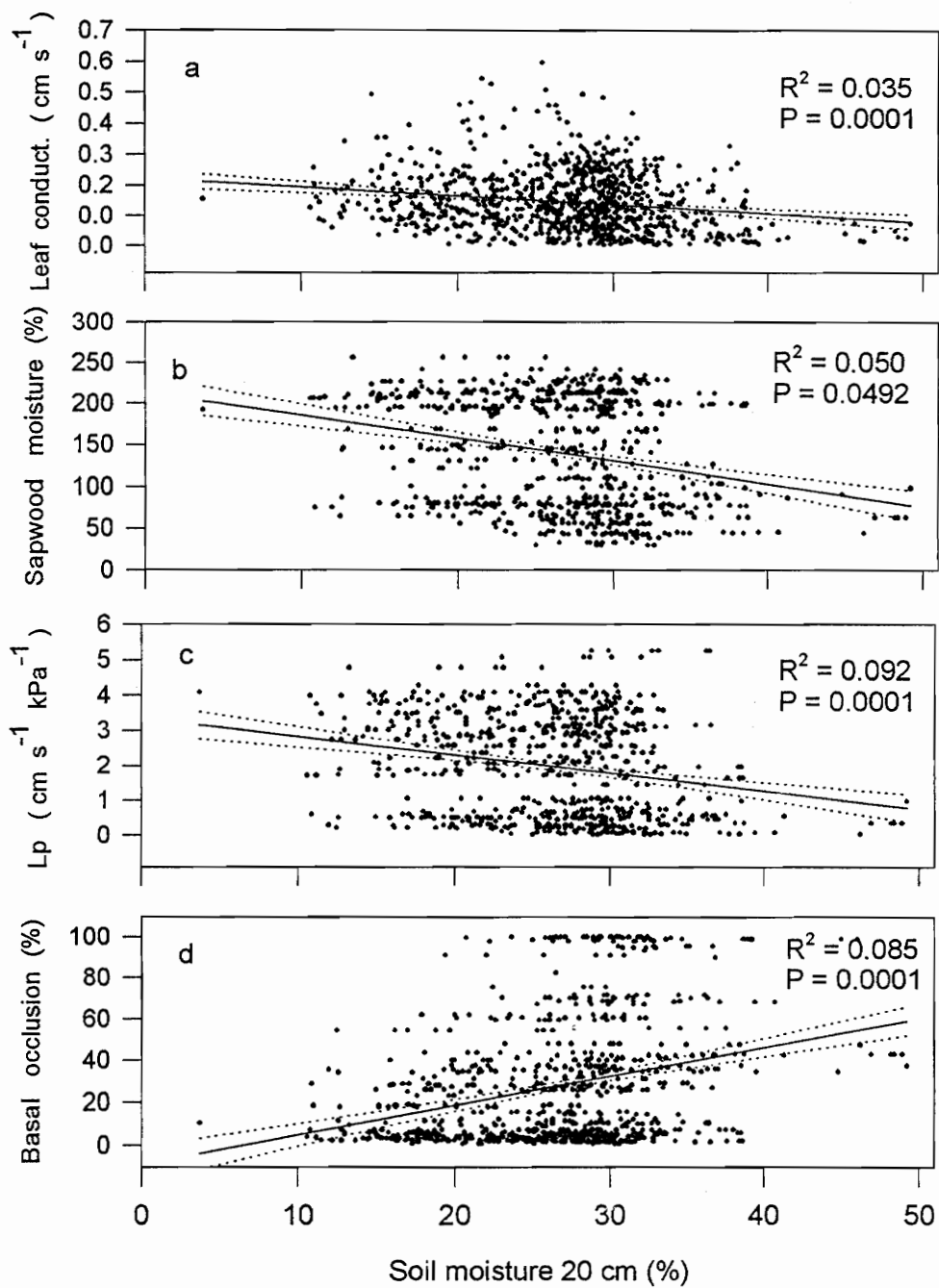


Figure 2.6. a, b, c, d. Relationship between disease severity variables and soil moisture at 20 cm (\pm 95% C.I.).

$P = 0.0001$) horizons and little colinearity with bulk density of the A ($r = -0.0755$, $P = 0.0001$) and B horizons ($r = -0.0831$, $P = 0.0001$). Stem Lp was significantly influenced by soil moisture at 20 cm, percent sand fraction of the B horizon, and percent slope (Table 2.8). There was a moderate degree of colinearity between soil moisture at 20 cm and percent silt fraction of the A horizon and percent slope ($r = -0.3498$ $P = 0.0001$). Soil moisture at 20 cm had the greatest impact on basal occlusion (Table 2.9). Percent slope also exerted a significant influence over basal occlusion, not surprisingly, soil moisture at 20 cm decreased as percent slope increased ($r = -0.3498$ $P = 0.0001$). Bulk density of the A and B horizons explained a small a small but significant amount of variation in basal occlusion. Only soil moisture at 20 cm and percent slope exerted significant influence over sapwood moisture content (Table 2.10).

Although soil moisture clearly exerted the greatest influence over disease severity it would be myopic to summarily discount the influence of the other significant soil-site factors. This study was designed to test the impact of soil moisture on PRD development and identify which microsite factors may contribute to moisture availability in Christmas tree plantations. Other significant variables included: percent slope, total porosity, textural classification and bulk density. The effect of slope on soil moisture is elementary; steeper slopes have lower soil moisture due to the nature of their drainage patterns. Soil moisture at 20 cm was correlated with total porosity of soil in the A ($r = 0.4623$ $P = 0.0001$) and B ($r = 0.4281$ $P = 0.0001$) horizons. Total porosity is defined as the proportion of the total soil volume not occupied by solid particles (Pritchett and Fisher 1987). Pore spaces are filled with air in dry soils, but in wet soils increased total porosity allows the increased retention of water. Particle

size also impacts the moisture content of soils. Finer particles (silt and clay) have more total pore space than coarse particles like sand (Brady 1984). Regression analysis of stem Lp identified the silt fraction of the A horizon as the most significant independent site variable. Correlation analysis showed that soil moisture at 20 cm and percent silt fraction were negatively correlated ($r = -0.3586$ $P = 0.0001$), and soil moisture and percent sand fraction were positively correlated ($r = 0.1344$ $P = 0.0001$). This seems to contradict previous statements made regarding total pore and water retention capacity. However there exists strong colinearity between the particle size variables. Before they were arcsin transformed, percent sand, silt and clay when added together equaled 100%, the reduction of one particle fraction invariably leads to the increase in another particle size fraction. The observed reduction in the silt fraction was linked to a complementary increase in both clay and sand particles.

In summary, this study demonstrated that expression of PRD symptoms in *P. strobus* is influenced by basal occlusion of the lower stem and the resulting reduction in sapwood hydraulic conductivity. The influence of site factors on disease severity and symptom expression was relatively small (stem Lp $R^2 = 0.211$, basal occlusion $R^2 = 0.131$, leaf conductance $R^2 = 0.127$, sapwood moisture content $R^2 = 0.116$), but highly significant ($P = 0.0001$). Of the significant soil variables influencing disease severity and symptom expression, elevated soil moisture at 20 cm appears to be the most important. In light of these results, I would recommend that Christmas tree growers avoid planting *P. strobus* on poorly drained sites.

Chapter 3

Influence of Soil Moisture on the Development of Procerum Root Disease Symptoms in Eastern White Pine (*Pinus strobus* L.) Seedlings.

INTRODUCTION

Procerum root disease (PRD) has been documented as a serious problem in eastern white pine (*Pinus strobus* L.) Christmas tree plantations in Virginia since 1980 (Lackner and Alexander 1982, 1983). Procerum root disease is caused by the fungus *Leptographium procerum* (Kendr.) Wingf., which is believed to be transmitted by the pales weevil *Hylobius pales* (Herbst) (Nevill and Alexander 1992b). Increased incidence of PRD has been linked to trees growing on poorly drained soils in low lying areas (Towers 1977, Shaw and Dick 1980, Sinclair and Hudler 1980, Halambek 1981, Smith 1991). The field studies (Chapter 2) assessed the physiological impact of PRD in white pine and its relationship to a wide range of soil-site factors. This study affords greater insight into the relationships between *L. procerum* colonization of host tissue, its physiological effect on the host and how abiotic soil and site factors may modify disease expression.

The greenhouse studies are intended to complement the field studies under more rigorously controlled conditions. One of the most confounding aspects of the field study was using trees that were already diseased, where the date of first inoculation and possible subsequent inoculations were unknown. Soil moisture content was impossible to control in the field, and selection of trees on differing drainage classes was the only way to engineer differences in soil moisture at any particular date. Greenhouse studies provide an excellent

opportunity to compare disease development across moisture classes under controlled conditions. An identifiable point of inoculation, uniform date of inoculation, and identical inoculation technique should allow development of a strong model to describe disease expression. Under these conditions, it should be possible to assess the influence of moisture class on the rate of *L. procerum* colonization. Regular monitoring of growing medium moisture with time domain reflectometry in conjunction with strict watering schedules make it possible to maintain distinct moisture classes in the greenhouse environment.

Results of previous seedling inoculation studies have been contradictory. Some scientists have reported rapid colonization and death of white pine seedlings after inoculation with *L. procerum* (Lackner and Alexander 1982, 1983). Others were unable to detect disease symptoms months after seedling inoculation (Wingfield 1986, Horner 1985, Carlson 1994). Carlson (1994) has suggested that PRD may not be a disease of seedlings. It is hypothesized that although seedlings may be inoculated with *L. procerum* they may not express disease symptoms for several years.

The objectives of this study are to quantify the influence of soil moisture and xylem conductivity on PRD symptom expression in artificially inoculated white pine seedlings and document morphological and physiological effects of PRD on white pine seedlings.

MATERIALS and METHODS

Plant Materials. Improved (2-0) eastern white pine seedlings were obtained in March 1994 from the Virginia Department of Forestry and planted in 3 liter pots, containing 2:1:1 peat/perlite/vermiculite medium. The seedlings were maintained in a cooled greenhouse

with natural light throughout the spring and summer. The pots were watered every 5-8 days depending on evapotranspirational water losses to avoid moisture extremes.

Inoculation Treatment. In early fall 26 weeks after planting, 78 seedlings were inoculated with *L. procerum* cultured on malt extract agar (MEA), 78 received MEA only. Seedlings were inoculated on the lower stem by removing a 5 mm circular plug of bark and cambium with a flame sterilized cork borer, affixing a 5 mm plug of inoculum and securing with parafilm® (Carlson 1994).

Moisture Treatment. Seedlings were watered as above for 2 weeks after inoculation. They were then divided into 26 blocks of 6 trees each in a randomized block design. Within each block, 2 trees (one fungal inoculated, one MEA inoculated) were randomly selected to receive one of three moisture treatments. Moisture content of the potting medium was maintained as follows for each treatment: 5-15% (droughty), 20-30% (optimum) and 35-45% (saturated). These values correspond to soil moisture variation observed at Christmas tree farms in Floyd County, Virginia (Chapter 2). Trees were watered every 14-20 days in the droughty treatment, every 5-7 days in the optimum treatment, and every day in the saturated treatment. Time domain reflectometry was used periodically to measure volumetric soil water content and make adjustments to the moisture treatments using a Trase System TDR (Model 6050X1, Soilmoisture Equip. Corp., Santa Barbara, CA). Fascicle water potential was measured with a pressure chamber (Model 1000, PMS Equipment Co., Corvallis, OR) (Scholander et. al. 1965) periodically to monitor the degree of water stress and make adjustments to the water treatments as needed.

During the initial nine weeks of water treatments, the seedlings were allowed to set

bud under natural fall photoperiods. Eleven weeks after inoculation, the seedlings were placed in a cold room at 4-5° C for a period of seven weeks to satisfy chilling requirements and ensure uniform budbreak. During the chilling period the trees were covered with plastic sheeting to prevent foliar desiccation from fans in the forced air cooler.

After chilling, the seedlings were moved back into a greenhouse and placed under a 16 hour photoperiod using artificial lights to extend daylight. They were exposed to temperatures of 18-22°C to simulate spring conditions and force bud break. The three water treatments were immediately resumed. The seedlings were maintained under these conditions until the termination of the study 36 weeks after inoculation.

Shoot Elongation Measures. The seedlings were monitored daily and the number of days to bud break was recorded. Once most of the seedlings had broken bud in mid-March, shoot elongation was measured periodically. The length of the terminal shoot was measured to the nearest half centimeter with a ruler at 27, 29, 31, and 36 weeks after inoculation. On May 23, 1995, one needle from each of five fascicles on the terminal shoot was measured to the nearest millimeter on each tree and mean needle length was calculated.

Water Relations Measures. On May 25, 1995, mid-day water potential was recorded for every tree using single detached fascicles. Stomatal conductance, transpiration, and diffusive resistance were also measured. Measures were made on single detached fascicles with a Li-Cor 1600 steady state porometer (Li-Cor, Lincoln, NE) fitted with a 4 cm² square closed system cuvette. To reduce variability in ambient light, an artificial light source was used to maintain PAR at 1800 $\mu\text{mol s}^{-1} \text{m}^{-2}$. A water filter was placed between the lamp and the seedling to prevent unnecessary heating. A small fan was attached beneath the water

filter to further dissipate heat. Measures were made between 11:30 am and 3:30 pm, one block at a time. The porometer was calibrated to a leaf area of 1 cm² and values were later corrected using the specific surface area of each fascicle. Surface area was computed with the following geometric relationship:

$$SA = 3.14159(d)(l) + (n)(d)(l)$$

Where d = fascicle diameter, l = needle length, and n = the number of needles in a fascicle (Ginn et al. 1991). The following morning, May 26, predawn water potential was measured between 3:30 am and 7:30 am. Trees in all treatments in 10 of the 26 blocks were then watered and mid-day and pre-dawn water potential were remeasured May 29 and May 30, respectively.

Growth Measures. The trees were harvested 36 weeks after inoculation. Current year's growth was removed from the seedlings and brought to the laboratory for biomass analysis. Average stem unit length (fascicles/cm) was recorded for the terminal shoot. To measure total root biomass, medium was shaken from the roots and they were rinsed free of debris with water. Needles, shoots, and roots were separated by hand and dried at 65° C to constant weight.

***L. procerum* Reisolation / Distance of Colonization.** Stem tissue above the point of inoculation was cut from the seedling with flame-sterilized shears and used for colonization studies. The cut stem was sprayed with 95% ethyl alcohol and flamed briefly for surface sterilization. Bark and cambium were peeled from the stem and the first 10 cm above the inoculation point was cut into 1 cm segments. Beginning 1 cm above the point of inoculation,

the stem wood was cut into 10 one cm segments. When sectioning bark and stem segments, cutting implements were flame sterilized before each cut. The bark and stem segments were kept in sequential order from the point of inoculation to facilitate measuring the distance of colonization from the point of inoculation. The ordered segments were placed on actidione malt agar (AMA) selective medium. The medium was prepared using a recipe slightly modified from McCall and Merrill (1980); 15.0 g agar, 15.0 g malt extract and 1 l of distilled water were combined in a 2 l flask. The medium was sterilized in a steam powered autoclave. Once the medium cooled to 55-60°C, 0.5 g actidione (cycloheximide) in 10 ml of 95% ethyl alcohol and 1.0 ml lactic acid (25%) were added. The medium was poured into sterile 15 cm disposable plastic petri plates, approximately 20 ml per plate. The plates were incubated at 21°C for 9 days and were read and rechecked between 9 and 14 days for the presence of *L. procerum*.

Hydraulic Conductivity Measures. Xylem hydraulic conductivity (L_p) was measured on the basal portion of each stem below the point of inoculation, using a technique originally described by Sperry et al. (1988). Hydraulic conductivity ($\text{cm s}^{-1} \text{kPa}^{-1}$) will be expressed in the following terms:

$$L_p = (\Delta J_v / \Delta P)(1 / A)$$

Where ΔJ_v ($\text{cm}^3 \text{s}^{-1}$) is the volume flow rate, ΔP (kPa) is the applied pressure and A (cm^2) is the lateral surface area (Nobel et al. 1990).

Constant water pressure to force water through the excised stem segments was created by placing a 20 l container at a height of 4 m with a rope and pulley. The water level

was maintained via a hose attached to the raised reservoir. This configuration created a pressure differential of 40 kPa. The pressurizing/flow control unit consisted of a closed end PVC pipe fitted to the raised water reservoir with several faucets to deliver water at a constant pressure to the stem segments. Stem segments were prepared by removing the tissue between the root collar and the point of inoculation on the stem. The stem was trimmed to a length of 5 cm under water and debarked. Still under water, the segments were inserted into a length of tygon tubing and sealed with parafilm. The open end of the tubing was attached to a faucet on the pressurizing unit. Water forced through the seedling stem was collected in a pre-weighed microcentrifuge tube at 1 minute intervals. The volume of water passing through the stem was measured by reweighing the centrifuge tubes at 1, 3, and 5 minutes after pressurization. In preliminary tests, peak flow was usually encountered in the first 3 minutes. Flow determination was accurate to 0.001 ml/min.

Data Analysis

All data were analyzed using SAS software on the Virginia Tech VM1 mainframe computer (SAS Institute, Cary NC). Simple mean separation analysis was accomplished using the general linear models procedure (GLM) with Tukey's HSD test ($\alpha = 0.05$). Tukey's HSD test was used to ascertain if there were any significant differences in mean distance of *L. procerum* colonization across the moisture treatments. The GLM procedure was used to determine if there was any interaction between the inoculation and moisture treatments for the following variables: predawn ψ , midday ψ , leaf conductance, transpiration, xylem hydraulic conductivity, number of days to bud break, stem units, needle length, dry mass of

new needles, dry mass of new stems, and total dry mass of roots. If there was no significant interaction between the treatments, means were separated by analyzing the treatments independently.

RESULTS

Inoculation observations. Seedlings inoculated with *L. procerum* and MEA (control) formed callus tissue around the point of inoculation. Callus tissue on seedlings inoculated with *L. procerum* was reddish in color and had more resin flow than the MEA controls. Some seedlings with *L. procerum* were swollen around the point of inoculation. *Leptographium procerum* was recovered from 19 of 78 inoculated seedlings and 0 of 78 of the MEA inoculated seedlings. Of the 19 trees infected, *L. procerum* grew 1 cm in 17 and 2 cm in only 2. There was no significant relationship between ability to reisolate *L. procerum* or distance of colonization, and the differential soil water treatments ($P=0.2512$).

Water relations measures. Inoculation with *L. procerum* had no effect on seedling water relations, while the soil water treatment strongly influenced water relations variables (Table 3.1). Seedlings in the saturated treatment exhibited the greatest leaf conductance followed by the optimum and droughty treatments (Table 3.2). Predawn water potentials of the saturated and optimum treatments were not significantly different, while the droughty treatment was more than twice as negative (Table 3.2). The optimum treatment had the greatest change in water potential from predawn to midday (-0.29 MPa), the droughty (-0.18 MPa) and saturated (-0.18 MPa) treatments exhibited significantly less change in water potential ($P=0.0003$). No interaction between the inoculation and moisture treatments was observed (Table 3.1).

Forty eight hours after the droughty treatment was re-watered and the saturated treatment was allowed to drain, differences in water potential among the treatments became

Table 3.1. Analysis of variance for seedling physiology variables 36 weeks after inoculation.

Source	df	Pre-dawn ψ		Mid-day ψ		Leaf conductance		Transpiration	
		F-value	p > F	F-value	p > F	F-value	p > F	F-value	p > F
Block	25	0.89	0.6185	1.28	0.1981	1.10	0.3580	1.02	0.4525
Inoculation	1	0.26	0.6145	0.07	0.7976	0.02	0.8902	0.05	0.8288
Inoculation x block	25	0.97	0.5167	1.05	0.4103	0.85	0.6702	0.90	0.6070
Moisture	2	115.84	0.0001	111.78	0.0001	116.65	0.0001	121.21	0.0001
Inoculation x moisture	2	0.14	0.8731	0.12	0.8898	0.30	0.7414	0.25	0.7787

Table 3.2. Water relations of 2-0 eastern white pine seedlings 36 weeks after inoculation.

Inoculation Treatment	Pre-dawn ψ (-MPa)	Mid-day ψ (-MPa)	Leaf conduct. (cm s^{-1})	Trans. ($\text{mmol m}^{-2} \text{s}^{-1}$)
<i>L.procerum</i>	0.89a ¹	1.10a	0.0955a	1.3247a
MEA	0.86a	1.09a	0.0947a	1.0342a
Moisture Treatment				
Droughty	1.35a	1.53a	0.0336c	0.478c
Optimum	0.65b	0.94b	0.1133b	1.590b
Saturated	0.62b	0.80c	0.1383a	1.932a

¹ Column means with the same letter are not significantly different using Tukey's HSD test ($\alpha = 0.05$).

Table 3.3. Analysis of variance for seedling physiology variables after droughty treatment was rewatered and daily watering of saturated treatment was suspended.

Source	df	Pre-dawn ψ		Mid-day ψ		Leaf conductance		Transpiration	
		F-value	p > F	F-value	p > F	F-value	p > F	F-value	p > F
Block	10	7.07	0.0349	16.06	0.0002	2.98	0.0068	4.13	0.0006
Inoculation	1	6.28	0.0456	24.06	0.0011	0.04	0.8418	0.04	0.8445
Inoculation x block	10	5.93	0.0481	17.01	0.0002	0.53	0.8611	0.48	0.8932
Moisture	2	0.59	0.5943	2.65	0.1308	33.47	0.0012	30.85	0.0001
Inoculation x moisture	2	0.12	0.8878	0.72	0.5137	2.99	0.0616	2.55	0.0904

Table 3.4. Water relations of (2-0) eastern white pine seedlings across soil water treatments after droughty treatment was re-watered and daily watering of the saturated treatment was suspended.

Inoculation Treatment	Pre-dawn ψ (-MPa)	Mid-day ψ (-MPa)	Stomatal Conduct. (cm s^{-1})	Trans. ($\text{mmol m}^{-2} \text{s}^{-1}$)
<i>L.procerum</i>	0.58a ¹	0.78a	0.0928a	1.4400a
MEA	0.53a	0.81a	0.0916a	1.4220a
Moisture Treatment				
Droughty	0.56a	0.65a	0.0603c	0.945c
Optimum	0.55a	0.80a	0.0990b	1.543b
Saturated	0.55a	0.85a	0.1174a	1.805a

¹ Column means with the same letter are not significantly different using Tukey's HSD test ($\alpha = 0.05$).

insignificant (Tables 3.3, 3.4). The saturated treatment continued to have the highest leaf conductance, followed by the optimum and droughty treatments (Table 3.4).

Xylem conductivity measures. There was no significant effect of the inoculation treatment on stem hydraulic conductivity (Table 3.5). Seedlings in the optimum soil water treatment exhibited the greatest conductivity, the saturated treatment was 32% lower, and the droughty treatment was 64% less (Table 3.6). No interaction between the inoculation and moisture treatments was observed (Table 3.5).

Seedling growth and biomass measures. The number of days to bud break was significantly influenced by the moisture treatment ($P=0.0001$) averaging 42, 45, and 49 days in the optimum, saturated, and droughty classes, respectively. Inoculation treatment had no effect on the number of days to bud break ($P=0.0710$), nor was there any significant interaction between the inoculation and moisture treatments ($P=0.6283$).

Trees inoculated with *L. procerum* had significantly greater shoot elongation on julian days 83, 96 and 107 than trees that received MEA inoculations; however, by the final measurement date the difference became insignificant (Figure 3.1). The soil water treatments had a significant impact on shoot elongation (Figure 3.2). Trees in the optimum treatment had the greatest shoot elongation (7.82 cm), followed by trees in the saturated (6.37 cm) and droughty treatments (3.69 cm) by the final sample date.

Seedlings in the optimum soil water treatment had accumulated the greatest growth by the end of the experiment. The optimum treatment exhibited the greatest needle length, new needle mass, new stems and total root mass (Tables 3.7). The low mean stem unit values indicate superior shoot elongation. Although seedlings in the saturated treatment had

Table 3.5. Analysis of variance for seedling xylem hydraulic conductivity.

Source	df	Hydraulic conductivity (cm s ⁻¹ kPa ⁻¹)	
		F-value	p>F
Block	11	0.45	0.9251
Inoculation	1	1.28	0.2649
Inoculation x block	11	0.91	0.5358
Moisture	2	5.87	0.0055
Inoculation x moisture	2	0.51	0.6033

Table 3.6. Xylem hydraulic conductivity of (2-0) eastern white pine seedlings as influenced by inoculation with *L. procerum* and soil water condition.

Inoculation Treatment	Hydraulic conductivity (cm s ⁻¹ kPa ⁻¹)
<i>L.procerum</i>	0.0494a ¹
Malt	0.0383a
Moisture Treatment	
Droughty	0.0233b
Optimum	0.0646a
Saturated	0.0438ab

¹ Column means with the same letter are not significantly different using Tukey's HSD test ($\alpha = 0.05$).

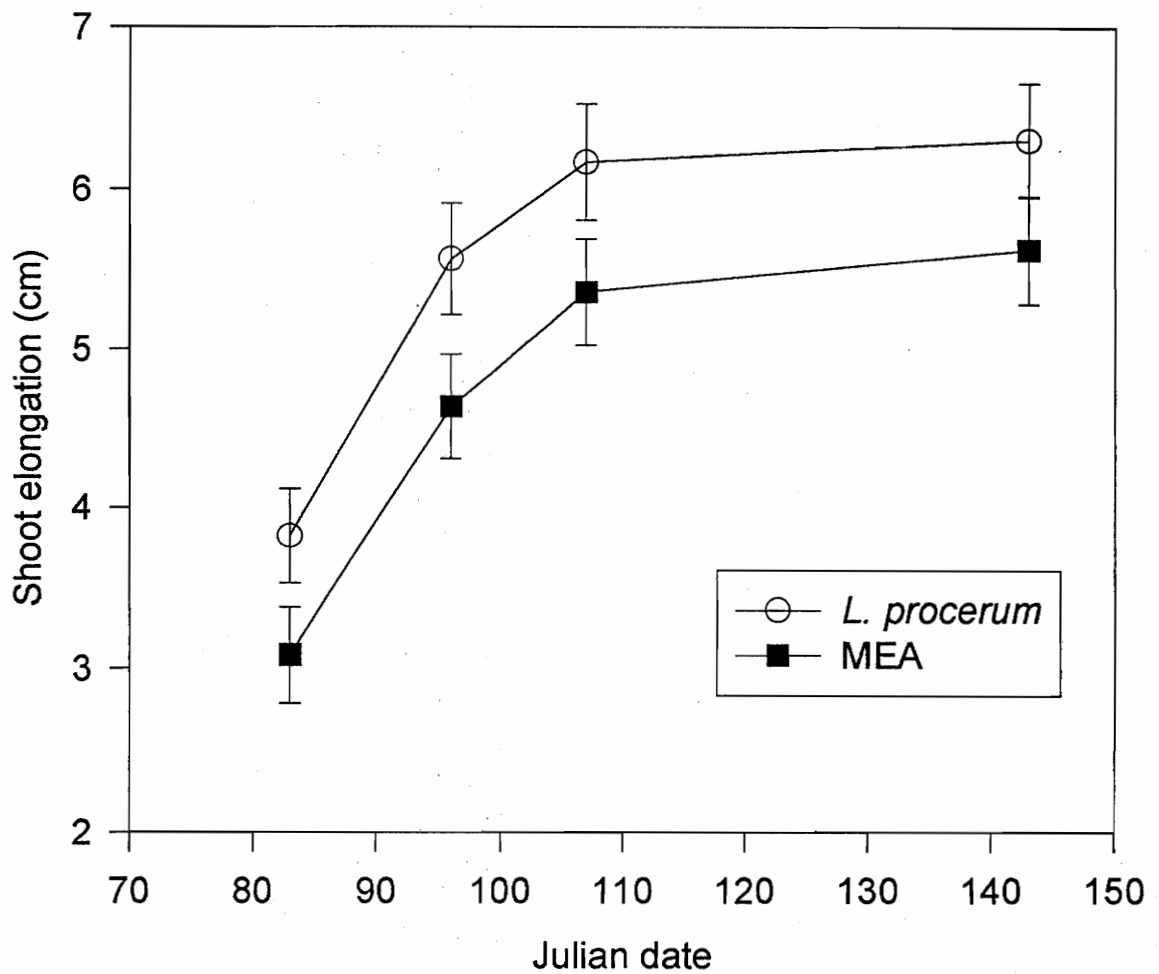


Figure 3.1. Mean cumulative shoot elongation of 2-0 *P. strobus* following inoculation treatments. Elongation in the *L. procerum* and MEA (control) treatments was found to be significantly different using Tukey's HSD test ($\alpha = 0.05$) at dates 83, 96, and 107, but not date 144. Error bars indicate \pm standard error of the mean.

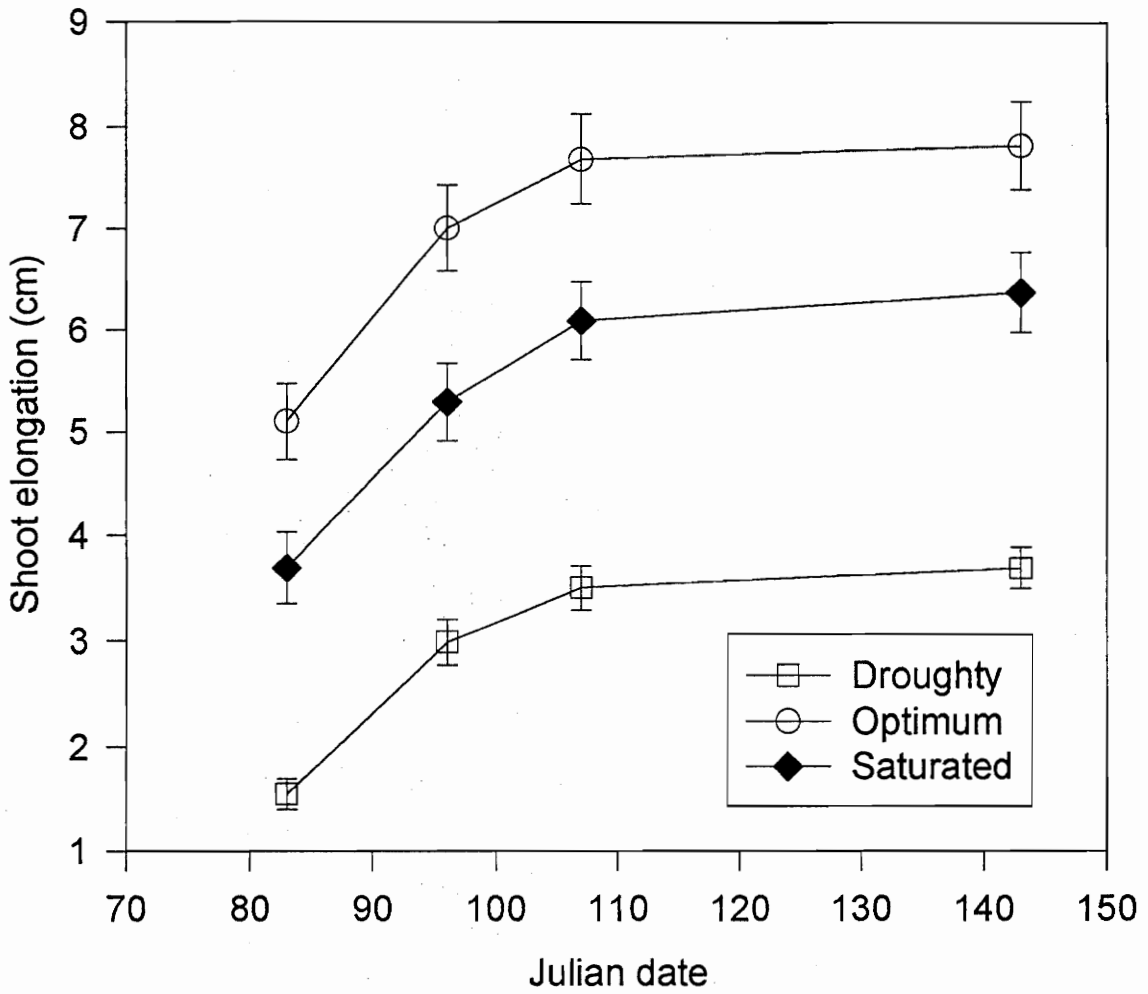


Figure 3.2. Mean cumulative shoot elongation of 2-0 *P. strobus* as influenced by watering treatment. Elongation in the droughty, optimum and saturated moisture treatments was found to be significantly different at all measurement dates using Tukey's HSD test ($\alpha = 0.05$). Error bars indicate \pm standard error of the mean.

Table 3.7. Biomass and morphology of 2-0 eastern white pine seedlings as influenced by *L. procerum* inoculation and soil water treatments.

Inoculation Treatment	Stem units (fascicles/cm)	Needle length (cm)	Mass of new needles (g)	Mass of new stems (g)	Mass total roots (g)
<i>L.procerum</i>	11.65a ¹	4.77a	2.10a	0.44a	7.77a
Malt	10.18a	3.87b	1.51b	0.36b	7.69a
Moisture Treatment					
Droughty	14.11a	3.61b	1.16c	0.21c	5.54c
Optimum	6.91b	5.14a	2.63a	0.58a	9.00a
Saturated	11.66a	4.21b	1.64b	0.41b	8.65b

¹ Column means with the same letter are not significantly different using Tukey's HSD test ($\alpha = 0.05$).

greater stomatal conductance (Table 3.2), they produced less biomass than the optimum treatment. Unexpectedly, needle length, new needle biomass and new shoot biomass were significantly higher in the *L. procerum* inoculation treatment (Tables 3.7, 3.8).

Table 3.8. Analysis of variance for seedling biomass and morphology variables

Source	df	Stem units		Needle length		Mass of new needles		Mass of new stems		Mass total roots	
		F-value	p > F	F-value	p > F	F-value	p > F	F-value	p > F	F-value	p > F
Block	25	0.73	0.8147	1.46	0.0979	1.03	0.4379	0.62	0.9311	0.77	0.7703
Inoculation	1	2.01	0.1589	15.51	0.0002	15.42	0.0002	4.56	0.0351	0.04	0.8416
Inoculation x block	25	1.05	0.4081	0.89	0.6236	1.29	0.1828	1.03	0.4706	0.72	0.8288
Moisture	2	16.2	0.0001	14.97	0.0001	32.88	0.0001	36.23	0.0001	28.25	0.0001
Inoculation x moisture	2	2.04	0.1349	0.24	0.7868	0.97	0.3809	0.71	0.4964	0.19	0.8247

DISCUSSION

This study was undertaken to elucidate the role of soil water stress on PRD development in *P. strobus* seedlings. Seedlings experienced dormancy induction, winter chilling and shoot elongation to ensure that they were exposed to any climatic factors or phenological stage that may enhance or encourage disease development. Despite these efforts, no interaction between inoculation and moisture treatments was observed for any of the experimental variables.

Leptographium procerum was recovered from only 24% of the fungal inoculated seedlings. Since the fungus was expected to grow at least 1 cm from the point of inoculation (Wingfield 1986), no plating was done closer to the inoculation point. It seemed unlikely that the fungus failed to infect in the 59 trees from which it could not be reisolated; it simply didn't grow at least 1 cm past the point of inoculation. In support of this, tissue from six extra trees remaining from the greenhouse experiment (five inoculated with *L.procerum* and one with malt agar) was macerated at the point of inoculation and plated on AMA medium July 9, 1995. *Leptographium procerum* was re-isolated from all five trees inoculated with the fungus and none was recovered from the tree with the MEA inoculation.

Leptographium procerum colonized host cambium tissue very slowly, growing less than 1 cm from the point of inoculation in most of the seedlings 36 weeks after inoculation. This colonization rate was much lower than reported by Wingfield (1986), who found the average distance of colonization of 8 isolates in eastern white pine seedlings to be 1.7 cm in 32 weeks. The soil water treatment had a strong influence on the development of seedlings,

but did not effect any changes in disease development or symptom expression in the *L. procerum* inoculated seedlings. Inoculation of seedlings with *L. procerum* had no negative effects on the morphological, physiological, growth or vascular variables studied. No seedlings died from *L. procerum* inoculation or displayed any symptom of PRD. Although there were significant effects of the soil water treatments on physiological, vascular, elongation and biomass variables and significant effects of the inoculation on elongation and biomass variables, there were no interactions between the two treatments. Both treatments exerted influence on the seedlings, but it is apparent that soil moisture did not impact disease severity as indexed by the physiologic and morphologic variables measured.

Many pathologists have associated increased incidence of PRD with poorly drained soils (Towers 1977, Shaw and Dick 1980, Sinclair and Hudler 1980, Halambek 1981, Smith 1991). It has always been unclear whether these observations were due to increased insect feeding causing multiple inoculation opportunities, or if PRD developed more rapidly and aggressively in moisture stressed trees. Goheen et al. (1978) found that 1-0 ponderosa pine (*P. ponderosa* Dougl. ex Laws.) seedlings inoculated with *L. wagneri* (Kendr.) Wingf., displayed increased infectibility and mortality in poorly drained soils. Although there was increased infectibility, there was no significant difference in distance of fungal colonization between seedlings in wet and dry soil treatments. Unfortunately Goheen et al. (1978) were unable to determine whether soil moisture was impacting the host, pathogen or both, leading to increased host infectibility. In my thesis studies, there were no negative effects of *L. procerum* inoculation on seedlings regardless of moisture treatment. *Leptographium wagneri* is a more typical root disease agent associated with definable disease centers and

soil borne fungal propagules that infect roots (Harrington and Cobb 1988). *Leptographium procerum* propagules have been isolated from soil immediately adjacent to diseased roots (Lewis 1985). However, the spores of *L. procerum* are short lived in soil and have limited efficacy, making them ineffective soil borne propagules (Lewis et al. 1987). Since there are few viable soil propagules to be invigorated by high moisture and infect moisture stressed roots, soil moisture may be a relatively unimportant factor affecting root infectibility.

The foliar symptoms of PRD: highly negative ψ , minimal daily change in ψ , reduced leaf conductance, poor shoot elongation and wilting can be likened to severe drought stress. Resin occlusion limits the quantity of water that can be delivered to the foliage and it is likely that trees growing on excessively drained soils would be at a further disadvantage. Droughty soil conditions have been linked to enhanced stem colonization of three pine species by *Sphaeropsis sapinea* (Bachi and Peterson 1985) and increased incidence of *Heterobasidion annosum* in loblolly pine (*P. taeda* L.) (Towers and Stambaugh 1968). In the seedling moisture manipulation study, there was no effect of the droughty treatment on PRD development.

There was a positive growth response of seedlings to *L. procerum* inoculation. Inoculated seedlings exhibited increased needle length, needle biomass and stem biomass. This response did not include the roots, whose biomass was almost identical for the two treatments. Positive growth responses to *L. procerum* inoculation have not been reported before. It is possible that fungus-induced growth regulators may play a role in the observed response. Significant amounts of gibberellins are produced in rice when infected with the fungus *Gibberella fujikuroi* causing bakanae disease (Bell 1981). Bakanae disease causes

rice seedlings to grow exceptionally tall. *Sphaceloma maniholicola* is another fungal pathogen which induces gibberellin production, causing superelongation disease of cassava (Zeigler et al. 1980). The subtlety of the growth increase associated with *L. procerum* contrasts sharply with the exaggerated growth found in bakanae disease of rice and superelongation of cassava. In trees, a variety of pathogenic organisms have been found to cause hypertrophic growth, mycoplasma-like organisms (MLOs) can induce witches' broom formation (Manion 1991). Many species of fungi, bacteria and parasitic plants cause root and stem galls in a myriad of forest tree species (Manion 1991). Unlike cankers and galls, the growth response observed in conjunction with *L. procerum* infection was an organized, differentiated growth. Biochemical assays for the presence of higher levels of gibberellins, cytokinins, auxins or other plant growth regulators would be necessary to explore this topic further.

Results of PRD studies on artificially inoculated seedlings conducted over the past 15 years have been contradictory at best. Using a root dip inoculation technique on two year old eastern white pine, Lackner and Alexander (1982) reported foliar chlorosis and wilt, dark staining of the xylem and seedling mortality two weeks after inoculation. Smith (1991) used a single, point inoculation technique, placing *L. procerum* inoculum below the bark surface on one year old eastern white pine, similar to the technique used in this experiment. Four weeks after inoculation, Smith observed chlorosis, wilt, and mortality and was able to reisolate *L. procerum* from symptomatic seedlings. These results portray *L. procerum* as a very aggressive, lethal pathogen of eastern white pine seedlings.

Other researchers using a variety of *L. procerum* isolates and inoculation techniques

have been able to successfully infect white pine, leading to the colonization of host tissue by the fungus (Horner 1985, Wingfield 1986, Carlson 1994). These researchers were able to re-isolate the fungus from seedlings, but they did not observe disease symptoms described by Lackner and Alexander (1982) or Smith (1991).

Several explanations for this dichotomy of results have been considered; 1) differences in inoculation technique and location of point inoculations, 2) loss of virulence in *L. procerum* isolates over time 3) minimum percent root infection required to induce disease development (Carlson 1994). Anecdotal information relating disease development in *P. strobus* to soil moisture extremes was not supported by this study. One of the more promising explanations for the lack of disease development in recent studies is that seedlings inoculated with *L. procerum* may not develop PRD until several years after inoculation. The pathogen may be effectively latent, until some host developmental stage is reached and susceptibility to PRD development changes (Nevill and Alexander 1992a, Carlson 1994). Long term seedling inoculation studies are ongoing at VPI & SU to address this hypothesis in the future.

There remains a very controversial possibility that *L. procerum* was not responsible for the symptoms observed by Lackner and Alexander (1982). We have never observed the symptoms reported by Lackner and Alexander on seedlings in the greenhouse or on seedlings outplanted in Christmas tree plantations where up to 80% of mature trees had died from PRD. Dark/black staining of xylem tissue of seedlings infected with *L. procerum* has not been observed in either greenhouse or field plantings in recent years. *Leptographium procerum* has been aptly described as weak wound pathogen, which colonizes seedling stem tissue very slowly and is unable to kill seedlings (Wingfield 1986). Possibly, *L. procerum* did not kill the

seedlings in the study by Lackner and Alexander. It seems more likely that some other pathogen such as *L. wagneri*, which colonizes eastern white pine seedlings rapidly, causes black staining, and rapid mortality was either misidentified as *L. procerum* or *L. procerum* inoculum was contaminated with another other pathogen.

In this study no mortality or symptoms developed in any of the seedlings inoculated with *L. procerum* regardless of water treatment. Work by Wingfield (1986) and Carlson (1994) also found no effects on seedlings; their work appears to be the most thorough and conclusive in the available literature. Considering their work and my results, I believe that PRD is not a seedling disease of *P. strobus*.

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VITA

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A handwritten signature in cursive script that reads "John Robert Butnor". The signature is written in black ink and is positioned centrally below the main text block.