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**PART TWO:**  
**FRONTIERS OF PLANT PHYSIOLOGY**

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**Influence of Procerum Root Disease  
on the Water Relations  
of Eastern White Pine (*Pinus strobus* L.)**

J. R. Butnor  
J. R. Seiler  
J. A. Gray

**INTRODUCTION**

Procerum root disease (PRD) is caused by the deuteromycete fungus *Leptographium procerum* (Kendr.) Wingf, formerly *Verticicladiel-*

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J. R. Butnor is affiliated with the Southern Research Station, USDA Forest Service, 3041 Cornwallis Road, Research Triangle Park, NC 27709 USA.

J. R. Seiler is affiliated with Virginia Polytechnic Institute and State University, Department of Forestry, Blacksburg, VA 24061 USA.

J. A. Gray is affiliated with the Virginia Polytechnic Institute and State University, Department of Plant Pathology, Physiology and Weed Science, Blacksburg, VA 24061 USA.

[Haworth co-indexing entry note]: "Influence of Procerum Root Disease on the Water Relations of Eastern White Pine (*Pinus strobus* L.)." Butnor, J. R., J. R. Seiler, and J. A. Gray. Co-published simultaneously in *Journal of Sustainable Forestry* (Food Products Press, an imprint of The Haworth Press, Inc.) Vol. 10, No. 1/2, 2000, pp. 95-105; and: *Frontiers of Forest Biology: Proceedings of the 1998 Joint Meeting of the North American Forest Biology Workshop and the Western Forest Genetics Association* (ed: Alan K. Mitchell et al.) Food Products Press, an imprint of The Haworth Press, Inc., 2000, pp. 95-105. Single or multiple copies of this article are available for a fee from The Haworth Document Delivery Service [1-800-342-9678, 9:00 a.m. - 5:00 p.m. (EST). E-mail address: [getinfo@haworthpressinc.com](mailto:getinfo@haworthpressinc.com)].

*la procera* (Kendr.) and is most commonly isolated from *Pinus* sp. L., though the fungus has been isolated from other conifer species including Fraser fir (*Abies fraseri* [Pursh] Poir.), Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) and Norway spruce (*Picea abies* [L.] Karst.) (Alexander et al., 1988). During the last two decades PRD has been responsible for significant economic losses in eastern white pine (*Pinus strobus* L.) Christmas tree plantations and trees in the urban landscape throughout the eastern United States (Lackner and Alexander, 1984).

Early studies reported rapid disease development in seedlings with mortality occurring 2 to 10 weeks after inoculation with *L. procerum* isolates (Lackner and Alexander, 1982). However, rapid disease development and seedling mortality have seldom been observed since. In Christmas tree plantations, white pines typically do not show any outward symptom of disease until they are near saleable maturity. The progression of PRD can vary greatly, but mortality usually occurs one or more years from the time the earliest symptoms are noted (Carlson, 1994). The initial symptoms of PRD in white pine include mild chlorosis, delayed bud break, and inhibited shoot elongation relative to healthy cohorts (Anderson and Alexander, 1979; Carlson, 1994). Resin exudation from the lower stem, basal stem irregularities due to localized cambium death, and basal stem 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 associated with mortality (Carlson, 1994). Observations of severe foliar symptoms usually occur in association with substantial resin soaking of sapwood tissue (Horner, 1987; Carlson, 1994). Resin soaked tissue exhibits reduced moisture content and greatly reduced permeability to water, caused by resinous inclusions that can completely block the tracheids (Horner, 1985, 1987). When the basal stem is sectioned, wedge-shaped areas of vascular occlusion that originate in the outer cambium and taper to the pith are frequently observed (Carlson, 1994).

Carlson (1994) developed a disease severity scale using a staining technique to quantify percent-occluded sapwood cross-sectional area at the base of the main stem. Pre-dawn water potential, daily change in pre-dawn to midday water potential, leaf conductance, transpiration and photosynthetic rate all showed trends toward lower values with increasing disease severity (Carlson, 1994). However, in selected

trees, physiological measures were not strongly correlated with disease severity. Some 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 probable a disease severity scale based on percent occluded basal area would not accurately reflect changes related to hydraulic conductivity of the sapwood, a variable not measured by Carlson (1994). Subtle declines in sapwood permeability to water may not be detected by direct observation (Horner et al., 1987), or aided with stain. Horner (1985) demonstrated that resinous occlusion limits water transport in white pine using small samples cored with an increment borer, but did not attempt to measure or describe the impact of resin soaking on a whole stem basis, where resin soaked sapwood can be adjacent to functional tracheids.

The purpose of this study is to quantify the effects of PRD on hydraulic conductivity of white pine sapwood using a whole stem measurement technique. This technique allowed us to assess the function of the vascular system and therefore, quantify total stem hydraulic conductivity by accounting for resinous occlusion, physical blockage of tracheids by fungal hyphae and resistance to flow in cavitated tracheids. We specifically tested the hypothesis that PRD reduces stem conductivity, which leads to reduced leaf conductance and eventually to the death of the tree.

## **MATERIALS AND METHODS**

### ***Study Sites and Sample Material***

Two Christmas tree plantations in Floyd County, Virginia were used for the study. Plantation 1 was under active cultivation and consisted of 8 ha of white pine and Scots pine (*P. sylvestris* L.) Christmas trees. Plantation 2 consisted of 20+ ha of white pine Christmas trees which had not been actively managed for 3-4 years. Heavy losses from PRD made this plantation economically nonviable. Four plots approximately 50 m in diameter were established at each plantation. Trees used for this study were near saleable maturity (7-9 years), were 2-2.5 m in height and measured 8-15 cm in diameter just above the root collar. In

June 1994, 12 visually symptomatic trees were selected for study in each of the eight plots. Every effort was made to choose 12 trees grading from barely to noticeably symptomatic. Mildly symptomatic trees displayed delayed bud break or retarded shoot elongation which 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. Six visually healthy control trees were located in the same plot interspersed with the diseased trees.

### *Gas Exchange*

Leaf conductance was measured with a Li-Cor 1600 steady state porometer fitted with a 4 cm<sup>2</sup> closed system cuvette (Li-Cor Inc., Lincoln, NE) (Bingham and Coyne 1977). Measurements were made periodically from July 15 to October 15, 1994, when two consecutive days of clear weather was predicted. (Due to the number of trees being sampled and the distance between plots, it was necessary to sample the plantations on two consecutive days.) Trees were sampled between 09:00 and 12:00 EST on clear to partly cloudy days with P.A.R. values ranging from 1500 to 2000, under ambient temperature and humidity. Measurements were made using one fascicle from each tree. Leaf conductance values were later corrected using the total surface area of each fascicle calculated using an equation presented by Ginn et al. (1991).

### *Hydraulic Conductivity*

At the end of the growing season, in late October, the sample trees were harvested for hydraulic conductivity analysis. Trees were cut as close to the ground as possible and returned to the laboratory. The stems were re-cut to remove any soil or sap accumulation on the cut surface. A 5 cm long segment was removed from the base of the stem, debarked and weighed. The segment was 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. Stem segments were fitted to the PVC pressurizing unit with a rubber gasket and the

unit was filled with water from a reservoir elevated 4 m. Pressure (40 kPa) from the raised reservoir was applied, forcing water through the stem segment. After a period of stabilization, lasting 5-10 minutes the volume flow rate was measured by collecting the water expressed through the segment in a one-minute interval. This was repeated every other minute until the readings stabilized. Healthy trees stabilized in 5 to 10 minutes, while diseased trees required up to 45 minutes.  $L_p$  ( $\text{cm s}^{-1} \text{kPa}^{-1}$ ) was calculated as:

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

Where  $\Delta J_v$  ( $\text{cm}^3 \text{s}^{-1}$ ) is volume flow rate,  $\Delta P$  (kPa) applied pressure difference and  $A$  ( $\text{cm}^2$ ) is the surface area (Nobel et al., 1990). After conductivity analysis, each segment was oven-dried at  $65^\circ\text{C}$  and reweighed to calculate initial percent moisture content (Panshin and de Zeeuw 1980).

### ***Basal Occlusion Measures***

After the 5 cm segments were removed for  $L_p$  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 band saw, revealing a pattern of green staining on the surface. Unobstructed vessels stained blue/green, while resin soaked and other dysfunctional vessels remained unstained (Basham, 1970; 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***

Data were analyzed using Proc GLM and Tukey's studentized range test (HSD) was used to determine significance of difference between the three disease classes (healthy, mildly symptomatic and diseased) and four physiological variables measured (leaf conductance,  $L_p$ , vascular occlusion and sapwood moisture content) (SAS Institute, 1991). A separate analysis was performed to describe the impact of disease

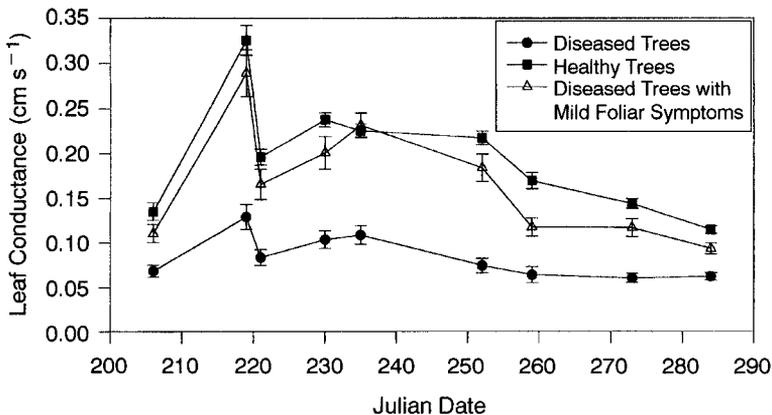
class by date on leaf conductance. Proc ANOVA was used to perform a one-way analysis of variance to determine the relation between  $L_p$ , vascular occlusion (%) and sapwood moisture content (%) and leaf conductance, using leaf conductance values from the last sample period immediately prior to harvest (SAS Institute, 1991). Regression analysis (Proc REG) was used to define the relationships among the measures of sapwood function ( $L_p$ , occlusion, sapwood moisture content) (SAS Institute, 1991). A total of 27 diseased trees died during the study and could not be analyzed for sapwood functionality.

## RESULTS AND DISCUSSION

### *Influence of Disease Class*

Procerum root disease has a profound effect on foliar and vascular physiology of white pine. Leaf conductance was significantly lower in diseased trees than healthy trees on each sampling date (Figure 1). The magnitude of the difference in leaf conductance stayed about the same throughout the course of the study. This indicates that the trees may be in a type of steady-state condition for an extended period, where PRD

FIGURE 1. Leaf conductance of healthy, diseased and diseased trees presenting mild foliar symptoms over the duration of the 1994 field study. Each data point represents the mean leaf conductance from all eight plots located on two different plantations  $\pm$  standard error of the mean data was collected on two consecutive days under similar weather conditions.



develops very slowly after reaching a developmental plateau. This is in line with Carlson (1994), who noted the development of PRD and PRD symptom expression usually preceded mortality by a year or more. Diseased trees with mild foliar symptoms were very similar to healthy trees in the first 30 days of measurement and the difference between the two classes was insignificant at two dates during this time (Figure 1). Later in the year, leaf conductance measured in the mildly symptomatic class remained consistently lower than measures in the healthy class. These mildly symptomatic trees are likely deviating from the healthy trees and exhibiting characteristics more consistent with diseased trees. Stem Lp and sapwood moisture content (%) were significantly higher in healthy trees, while mean vascular occlusion was greatest for the diseased trees (Table 1). Infection and colonization of host sapwood by *L. procerum* induces the host to fill affected regions with resins to limit fungal colonization (Horner, 1987). Under most circumstances this would be an effective strategy to slow or repel invading insects or fungal pathogens; however, *L. procerum* has the ability to survive and colonize resin-soaked woody tissue (Horner, 1985). Instead of limiting fungal colonization, the host is systematical-

TABLE 1. Results of GLM procedure defining relationship between disease class and water relations variables in white pine. Lp, occlusion (%), and sapwood moisture (%) were measured destructively over a two week period beginning in late October. Only leaf conductance measures from the final sample period (Day 284 and 285) just prior to harvest were used for this analysis.

Disease Class	n	Lp ( $\text{cm s}^{-1} \text{ kPa}^{-1}$ )	Occlusion (%)	Sapwood Moisture (%)	Leaf Conductance ( $\text{cm s}^{-1}$ )
Healthy	48	3.20 a <sup>2</sup>	4.16 a <sup>3</sup>	208.72 a	0.01153 a
Mildly Symptomatic	14	2.27 b	15.32 a	145.39 b	0.0938 a
Diseased	55	0.51 c	47.77 b	72.32 c	0.0625 b
R <sup>2</sup>		0.39	0.76	0.64	0.90
P-value		0.0001	0.0001	0.0001	0.0001

1. For date 284 and 285 only

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

3. Healthy trees do not truly have occluded sapwood. The technique used includes the small amount of heartwood these trees form as a portion of the stem that is not functional.

ly reducing its ability to conduct water. Sapwood moisture content had the best correlation with disease class ( $R^2 = 0.90$ ,  $p = 0.0001$ ) followed by  $L_p$  ( $R^2 = 0.76$ ,  $p = 0.0001$ ) (Table 1). Occlusion ( $R^2 = 0.64$ ,  $p = 0.0001$ ) and leaf conductance ( $R^2 = 0.39$ ,  $p = 0.0001$ ) were unable to delineate between mildly symptomatic and healthy trees.

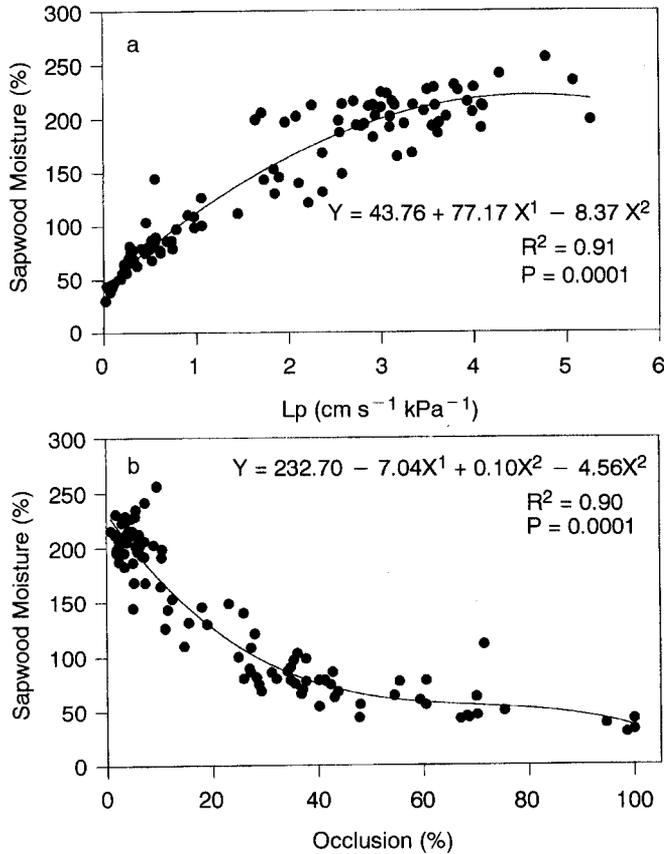
### *Relationship of Leaf Conductance to Stem Hydraulic Properties*

Leaf conductance was positively correlated with stem  $L_p$  and sapwood moisture content and inversely related to vascular occlusion, (Table 2), indicating that decreased leaf conductance is an expression of increasing disease severity. However, much of the variation in leaf conductance is not explained by either disease class or the vascular variables and is likely due to a variety of external variable that can influence stomatal function (Tables 1 and 2). The reduced leaf conductance is likely caused by the reduced capacity to conduct water, caused by resin infiltration of tracheids. As water-conducting vessels are filled with resin and rendered non-functional, moisture content of the sapwood is reduced (Figure 2 a, b). It was surprising that  $L_p$  had a lower correlation with leaf conductance than with occlusion given that  $L_p$  was more closely related to disease class. This is likely due to the inherently high degree of variability in the  $L_p$  measurements. Measurement of  $L_p$  is difficult, time consuming, destructive and explained less of the variation in leaf conductance than vascular occlusion, but did quantify the effect of occlusion on stem physiology in white pine. Regression analysis revealed a strong relationship between basal occlusion and stem  $L_p$  (Figure 3). As basal occlusion increased, making tracheids impermeable to water, stem  $L_p$  dropped markedly. When the

TABLE 2. ANOVA results defining the influences of  $L_p$ , occlusion (%), and sapwood moisture (%) content on leaf conductance in white pine. Only leaf conductance measures from the last sample date before harvest were used.

Stem Measure	Leaf Conductance				
	n	R <sup>2</sup>	Intercept	Slope	P-value
$L_p$	117	0.36	0.0575	0.0166	0.0001
Occlusion (%)	117	0.48	0.1155	-0.011	0.0001
Sapwood Moisture (%)	117	0.46	0.0323	0.0004	0.0001

FIGURE 2. Relationship between  $L_p$ , occlusion (%), and sapwood moisture (%) defined using (a) 2nd order and (b) 3rd order linear regression analysis ( $n = 117$ ).

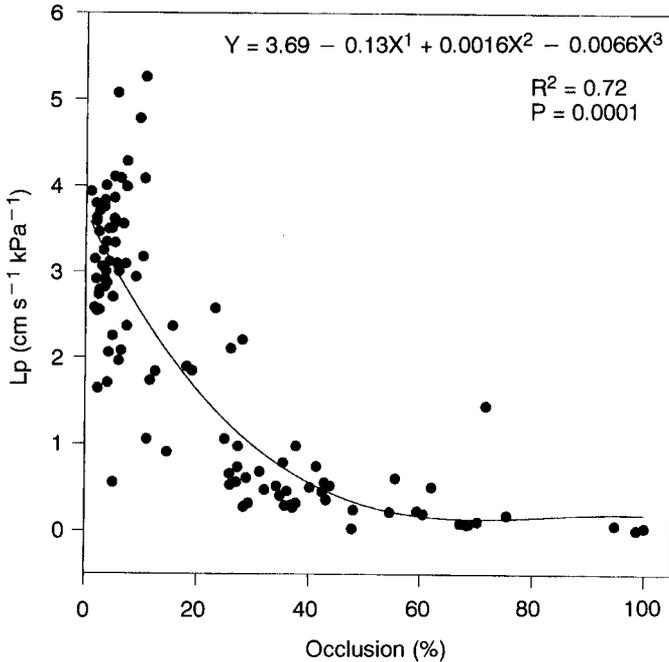


stem segments exhibited 20% occlusion, stem  $L_p$  was reduced over 50% and stem  $L_p$  approached zero at basal occlusion greater than 50%. From these results it appears that foliar disease expression is simply a matter of fluid dynamics, where water available to the leaves is lower and the leaves respond accordingly.

### Summary

Diseased trees exhibited greater vascular occlusion, lower sapwood moisture content, and reduced hydraulic conductivity; however, these

FIGURE 3. Results of 3rd order linear regression analysis (n = 117) describing the relationship between measures of sapwood occlusion and Lp.



variables were only moderately correlated with leaf conductance. The blockage of tracheids with resin was previously shown to make sapwood less permeable to water using small sections. We have demonstrated using a whole stem measurement technique that vascular occlusion resulting from PRD reduces stem hydraulic conductivity leading to reduced leaf conductance. The amount of water conducted through the stem is reduced resulting in the desiccation of the sapwood and foliage leading to premature mortality in eastern white pine.

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