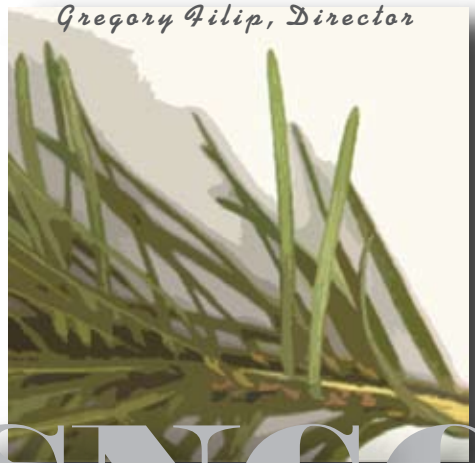
November 2001

Swiss Needle Cast Research Cooperative

Annual Report 2001

Gregory Filip, Director



SNCC

OREGON STATE UNIVERSITY
COLLEGE OF FORESTRY

Oregon State UniversityMembers of the Swiss Needle Cast Cooperative and Their 2001 Contributions

Boise Cascade Corporation	$22,500
Confederated Tribes of the Grand Ronde	$7,500
Confederated Tribes of the Siletz	$700
Hampton Resources, Inc.	$22,500
John Hancock Life Insurance	$22,500
Longview Fibre Co.	$22,500
Menasha Corporation	$22,500
Miami Corporation	$7,500
Oregon Department of Forestry	$22,500
Rosboro Lumber Co.	$7,500
Simpson Timber Co.	$22,500
Starker Forests	$22,500
Swanson Superior Forest Products, Inc.	$7,500
The Timber Company	$22,500
Weyerhaeuser Corporation	$22,500
Willamette Industries	$22,500
USDA Forest Service	In kind
USDI Bureau of Land Management	$22,500
OSU Forest Research Laboratory (salary) Swiss Needle Cast Cooperative	$30,000

Swiss Needle Cast Cooperative

Annual Report 2001

Edited by Greg Filip, SNCC Director

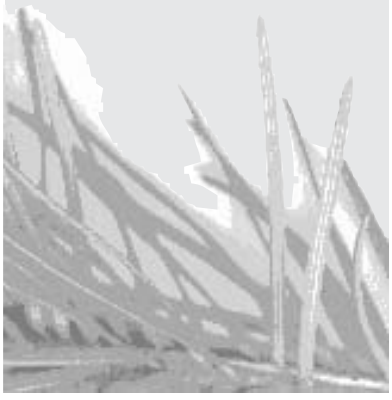
Layout by Gretchen Bracher, FRL Publications

SNCC income sources and expenditures 2001

Income	
Membership Dues	$300,700
Oregon State Legislature	$120,000

Expenditures (as of 8/01)	
Salaries and Wages	$159,783
OPE	45,828
Supplies and Services	116,714
Travel	16,830
Indirect Costs	14,067
Total Expenditures	$353,222

Contents



To: SNCC Members
From: Greg Filip
Date: September 2001
Subject: 2001 Annual Report

SNCC is now five years old, and I thank the members for all of the support that they have given SNCC this year. For two years we were fortunate to receive $240,000 from the Oregon State Legislature to support projects for 2000 and 2001. This year's annual report contains summaries on the progress made on our 12 projects. We had an aerial survey this year in Oregon that shows a continuing but apparently decreasing infestation of Swiss needle cast. Information continues to be collected on the permanent growth impact and precommercial thinning plots. Progress continues on the basic infection biology research that is summarized in this report. Projects are continuing in needle physiology, soil and foliage nutrition, and tree genetics, and progress reports are contained in this report. Several publications concerning SNC were written this year based on results obtained through SNCC.

I would like to especially thank this year's project investigators for their fine efforts in generating new information concerning Swiss needle cast: Alan Kanaskie, Doug Maguire, Katy Kavanagh, Jeff Stone, Dan Manter, Randy Johnson, Scott Ketchum, Floyd Freeman, Robin Rose, Cathy Rose, and Gary Chastagner. And thanks to the many graduate students and research assistants who do so much of the work, Lori Winton, Pablo Rosso, Paul Reeser, Wendy Sutton, Gabe Crane, and Fatih Temel. I would also like to thank the members of the SNCC executive committee who's enthusiasm and creativity keep this cooperative moving in the right direction: Mark Gourley, Tim Tompkins, John Trobaugh, Greg Johnson, Dale Claassen, Jim Carr, Mari Kramer, Will Littke, and Alan Kanaskie. We have at least 9 projects planned for 2002; it should be another exciting and productive year.

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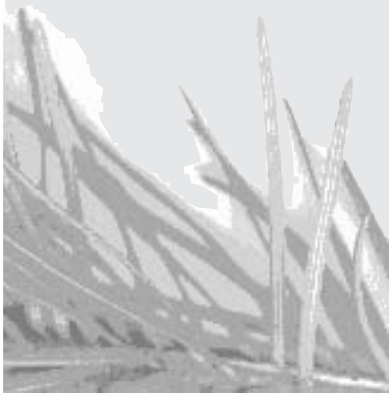
Highlights of 2001

This report presents the Swiss Needle Cast Cooperative activities in Swiss needle cast research. Highlights for 2001 include:

- An aerial survey was conducted over 2.9 million acres in western Oregon. A total of 221,000 acres of Douglas-fir had obvious symptoms of Swiss needle cast. In general, symptoms of Swiss needle cast decreased in 2001 compared to 2000. Survey maps can be obtained from Alan Kanaskie, Oregon Department of Forestry in Salem.
- A series of permanent plots were established by Floyd Freeman, BLM, in the Cascade foothills on private and public land to monitor for SNC severity and impacts.
- Research continues on 12 different projects in 2001 including: aerial survey, growth impact studies, tree physiology, infection biology, tree genetics, alternative fungicides, precommercial thinning, nutrient trends, nutrient imbalances, sulfur/tree growth, sulfur efficacy, and fertilizer and vegetation control.
- Dan Manter and Lori Winton completed their PhD dissertations: "Physiological impacts of Swiss needle cast on Douglas-fir" and "Phylogenetics, population genetics, molecular epidemiology, and pathogenicity of the Douglas-fir Swiss needle cast pathogen *Phaeocryptopus gaeumannii*."
- Dan Manter, Barbara Bond, Katy Kavanagh, Pablo Rosso, and Greg Filip published their research in the journal "New Phytologist" with the title "Pseudothecia of Swiss needle cast fungus, *Phaeocryptopus gaeumanni*, physically block stomata of Douglas-fir, reducing CO_2 assimilation."

Plans for 2002

- Continue aerial and ground survey to monitor SNC severity in coastal Oregon
- Continue to monitor permanent plots in the Cascade foothills
- Continue to monitor permanent plots from the growth impact study Phase III
- Continue to monitor precommercial thinning plots in stands with varying intensity of SNC in the Coast Range
- Continue studies on stem and root limitations to water movement in SNC-affected trees, and effects of microclimate on fungal development and physiological impact.
- Continue with infection biology studies: factors affecting colonization rate and foliage retention; *P. gaeumannii* infection, development, and reproduction; aerobiology and epidemiology; population biology of *P. gaeumannii;* and fungicide control studies.
- Determine tree responses to experimental alteration of nutrient availability across a gradient in SNC severity
- Continue studies on nutritional imbalance as a predisposing factor to SNC
- Determine impacts of SNC on wood quality of Douglas-fir
- Continue to monitor the effects of fertilization and vegetation control on SNC infection and growth of coastal Douglas-fir



Background and Organization

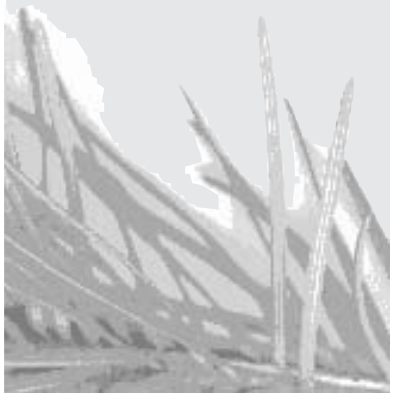
The Swiss Needle Cast Cooperative (SNCC) was established in January 1997. Damage caused by Swiss needle cast, a native foliage disease that affects Douglas-fir, has made it imperative that new research be conducted to learn practical methods of disease detection and management to maintain the health and productivity of Douglas-fir plantations. A well-run cooperative is an efficient means of increasing and accelerating the level of forest disease research in the region. Because members participate directly in problem identification and research solutions, communications of results is speeded and results are applied more rapidly and effectively.

SNCC is located in the College of Forestry at Oregon State University. The Membership is comprised of private, county, state, and federal organizations. Membership dues vary depending on forestland ownership. One annual report, project reports, and newsletters are distributed to members each year. All projects are carried out in cooperation with specific members on their land holdings.

Purpose

The focus of SNCC will be Swiss needle cast research for forestland owners in western Oregon and Washington. The purpose of SNCC is to provide the following services:

1. Conduct research on the biology, detection, and management of Swiss needle cast in coastal Douglas-fir as related to basic infection biology and genetics, tree physiological dysfunctions, aerial and ground survey technology, disease hazard and risk rating, growth and yield impacts, and strategies for control.
2. Conduct training and workshops on research and survey results
3. Provide newsletters and reports on research and surveys, and
4 Serve as a focal point for information on Swiss needle cast.



Swiss Needle Cast Aerial Survey, 2001

Alan Kanaskie, Mike McWilliams, Keith Sprengel, and Dave Overhulser

Survey procedures:

The observation plane flew at 1,500 to 2,000 feet above the terrain, following north-south lines separated by 2 miles. Observers looked for areas of Douglas-fir forest with obvious yellow to yellow-brown foliage, a symptom of Swiss needle cast. Patches of forest with these symptoms (referred to as polygons) were drawn onto 1:100,000 scale topographic maps. Each polygon was classified for degree of discoloration as either "S" (severe) or "M" (moderate). Polygons classified as "S" for discoloration had very sparse crowns and brownish foliage, while those classified as "M" were predominantly yellow to yellow-brown foliage and slightly more dense crowns than those classified as "heavy".

For the second year in a row, observers used a computer-based sketch-mapping system linked to a real-time Geographic Positioning System (GPS). Observers recorded damage onto computer touch screens that displayed topographic maps and the position of the aircraft. This technology allowed observers to spend less time navigating and more time mapping, and generally increased precision and accuracy compared to previous surveys using paper sketch maps.

The Coast range was surveyed on May 3, 7, 8, 9, and 10, 2001. It extended from the coastline eastward until obvious symptoms were no longer visible, and from the Columbia river south to Brookings. The west slope of the Cascade range was surveyed from the Columbia river south to Roseburg on May 8, 2001. We experienced almost no delays due to weather.

Results and discussion:

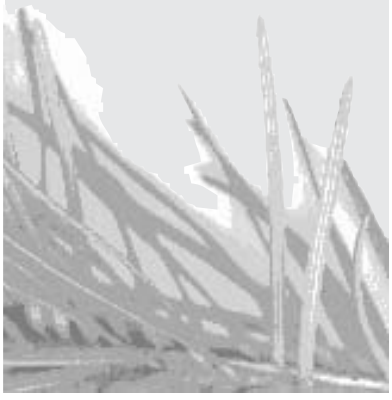


Figure 1 shows the approximate size and location of areas of Coast range Douglas-fir forest with symptoms of Swiss needle cast detected during the survey conducted in 2001. Figures 2 - 6 show survey results for 2000-1996.

The Coast Range survey covered about 2.9 million acres of forest. Approximately 221,000 acres of Douglas-fir forest had obvious symptoms of Swiss needle cast: 160,000 acres north of Florence, and 61,000 acres south of Florence. This is a decrease of about 62,000 acres compared to the 2000 survey (table 1, figure 7). Most of the decrease in the number of acres with symptoms occurred in Lincoln and Tillamook counties.

South of Florence, the number of acres with symptoms increased by about 2,000 acres between 2000 and 2001. Trends for individual counties appear in figure 8. The easternmost area with obvious SNC symptoms was almost 35 miles inland from the coast, which is a slight increase compared to previous surveys. Most of the areas with symptoms that can be detected from the air occurred within about 18 miles of the coast.

No Swiss needle cast damage was mapped in the Cascades, although Swiss needle cast does occur at damaging levels in some areas. Thus far the disease appears to be of concern only in a few localized areas.

The results of the 2000 and 2001 surveys suggest continuing intensification of SNC south of Florence, and a decrease in the area with symptoms in the north coast region. Some of this decrease could be due to the inherent variability of symptom development from year to year, the use of the new sketch mapping technology, and conversion of severely damaged stands. In some cases the disease can be so severe that detection is difficult

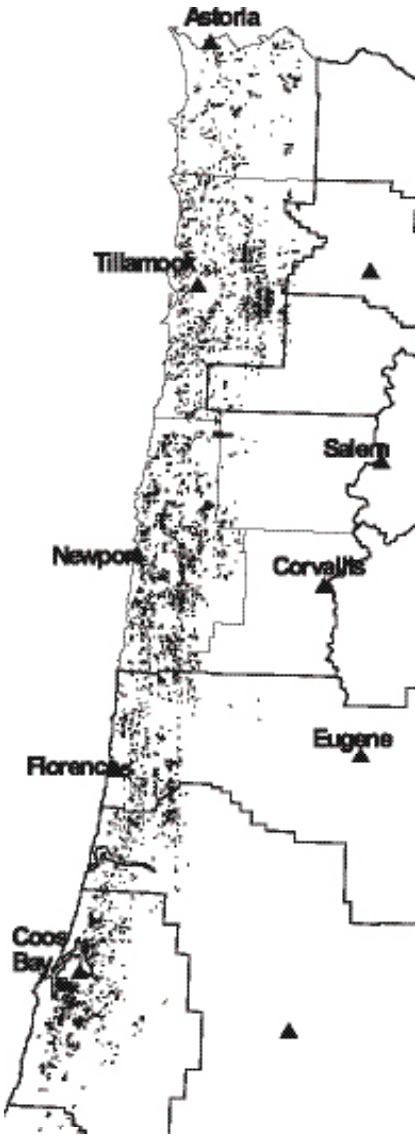


Figure 1. *Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May,* ***2001****.*

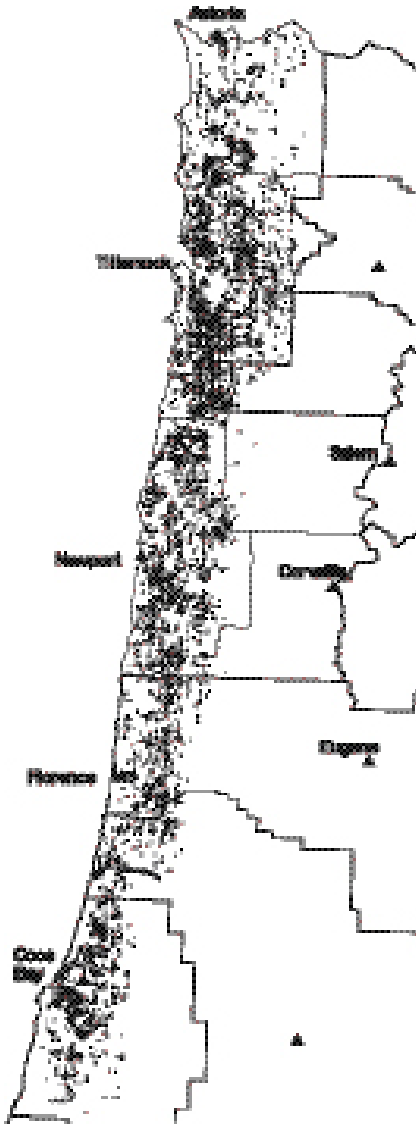


Figure 2. *Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May,* ***2000****.*

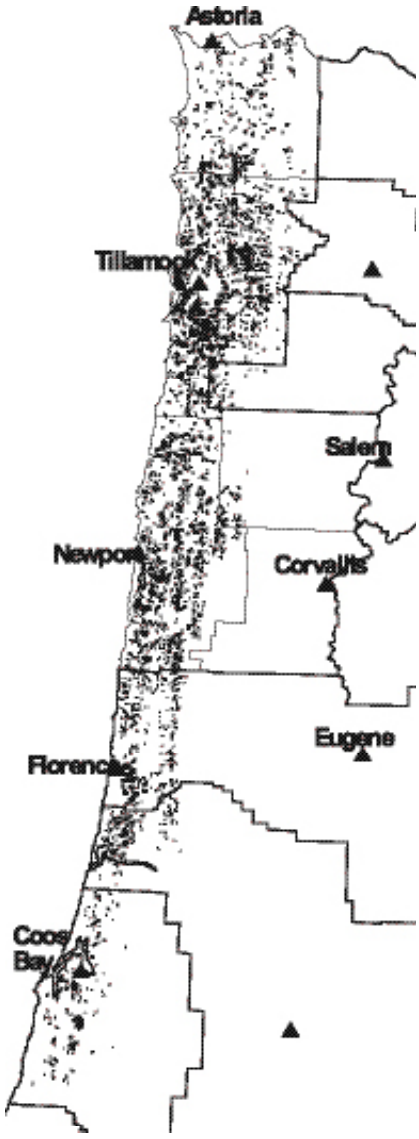


Figure 3. *Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May* ***1999****.*

Table 1. Area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in 1996-2001.

Region	1996	1997	1998	1999	2000	2001
	acres					
North of Florence	106,000	130,000	135,000	259,000	226,000	160,000
South of Florence	24,000	30,000	38,000	36,000	57,000	61,000
TOTAL	30,000	160,000	173,000	295,000	283,000	221,000

because of lack of foliage. Because symptoms develop rapidly during April and May, later surveys usually detect more areas with symptoms than surveys conducted earlier. The 2001 survey was completed relatively quickly during the preferred time window for symptom detection. Ground observations after the survey flights confirmed that even though tree phenological development (as indicated by bud break) was about 10 days

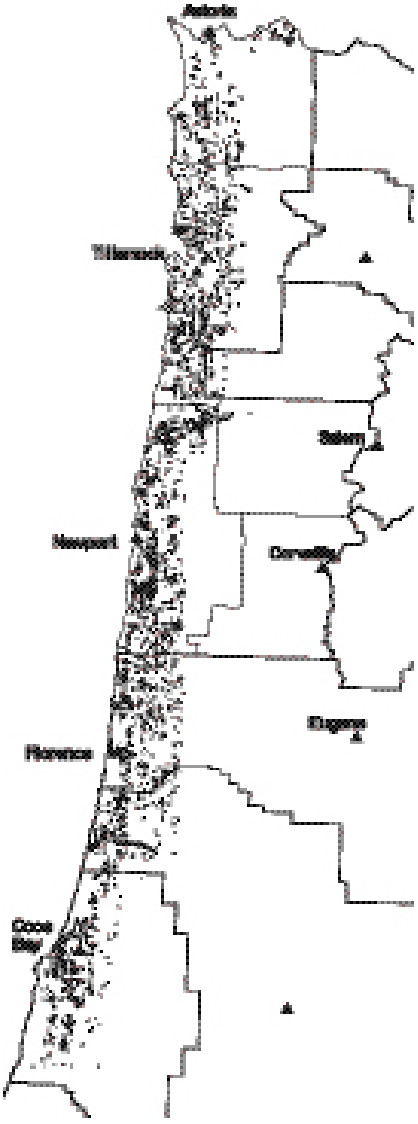


Figure 4. *Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May,* ***1998.***

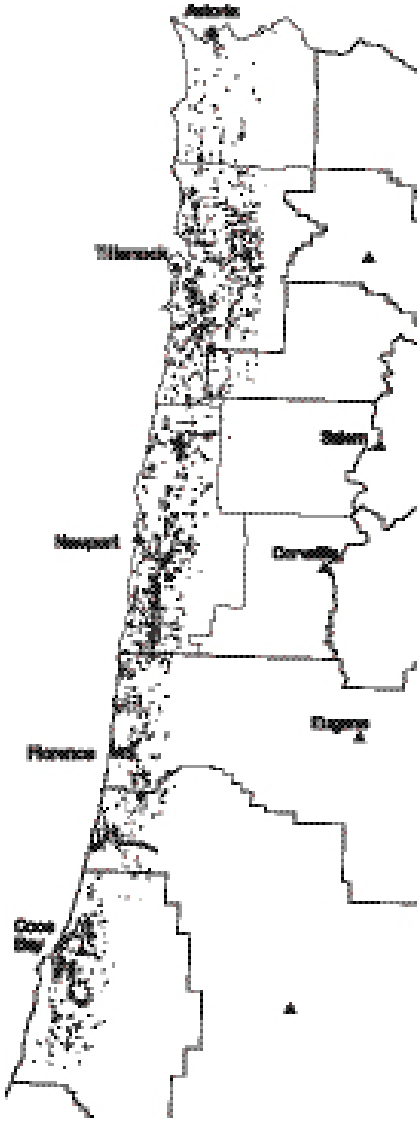


Figure 5. *Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May,* ***1997*** *(does* ***not*** *include re-fly of Nehalem and Yamhill quadrangles).*

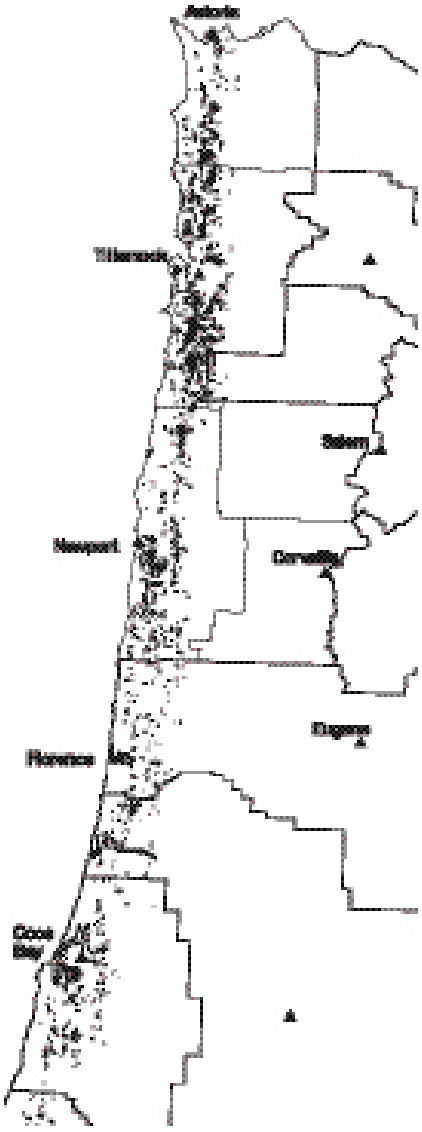


Figure 6. *Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April,* ***1996.***

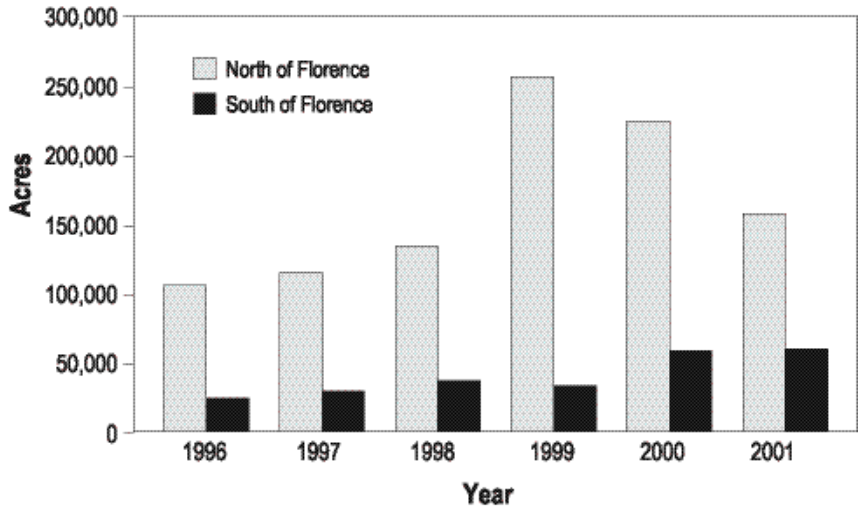


Figure 7. *Trend in area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in April and May, 1996-2001.*

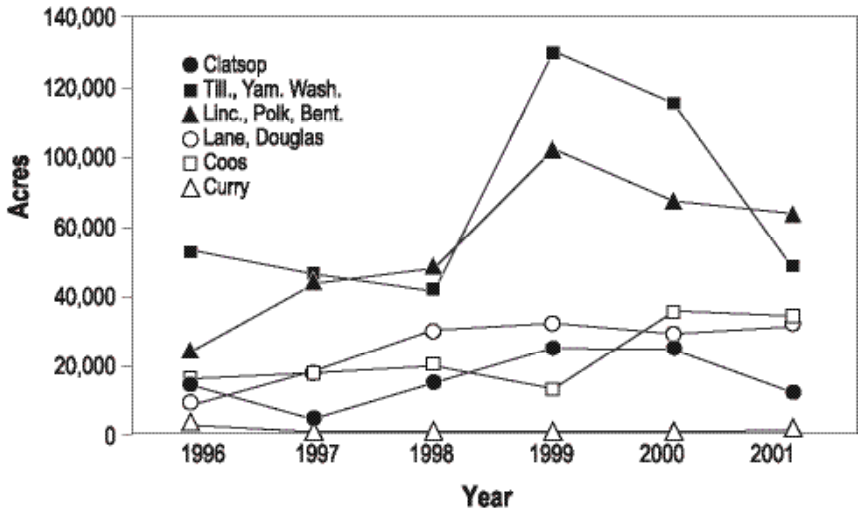


Figure 8. *Area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in April and May, 1996-2001, by county.*

later than average, symptoms were well-developed during the survey, but probably not at their peak.

Aerial survey results are conservative estimates of damage because observers map only those areas where disease symptoms have developed enough to be visible from the air. Permanent monitoring plots and ground checks have shown that Swiss needle cast occurs throughout the survey area, but that symptoms often are not developed enough to enable aerial detection. Because the survey detects discoloration and does not describe needle retention (which is correlated with growth loss), estimates of disease impact on tree growth should not be made from the aerial survey alone.

The aerial survey does provide a broad picture of the area significantly impacted by Swiss needle cast. From a practical standpoint, it establishes a zone in which forest management should take into account the effects of the disease.

Acknowledgments

The survey was conducted by the Oregon Department of Forestry Insect & Disease and Air Operations sections, and was funded by the USDA Forest Service Forest Health Monitoring Program and the Oregon Department of Forestry. Jack Prukop (ODF) piloted the plane. Mike McWilliams (ODF), Keith Sprengel (US Forest Service), and Dave Overhulser (ODF) were the aerial observers.

Swiss Needle Cast Monitoring Transects in the Oregon Cascade Range

Floyd Freeman, Salem BLM

Cooperators: Swiss Needle Cast Coop-directed field crews and SNCC staff.

Objective

To provide baseline information on Swiss needle cast (SNC) in selected stands in the Cascades and monitor SNC levels on an annual basis for up to 5 years.

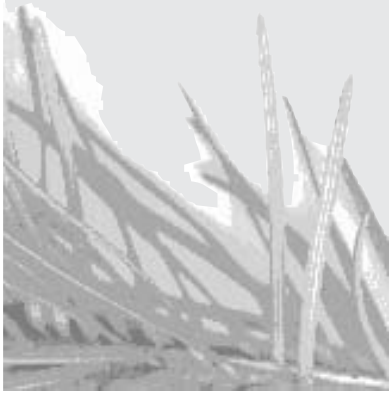
Importance and History: In addition to the large SNC infestations in the Oregon Coast Range, the Cascades Range in Oregon is also experiencing moderate SNC levels. Washington's Coast and Cascade Ranges also are infected as are areas in northern Idaho.

Project Objective

Install sampling transects in 60 stands (one transect per stand) according to SNC protocol developed by Alan Kanaskie and Doug Maguire. These transects will be comparable to the same grid established in the Oregon Coast Range in both numbers of stands sampled and the area sampled. Stands to be sampled will be 10 to 20-years old and more than 50 % Douglas-fir.

The area to be sampled ranges from the Columbia Gorge south to Oakridge. We stopped monitoring at Oakridge for climatic and logistical reasons. There is a large rain shadow ridge in this area where the climate is much dryer than to the north; the western hemlock zone begins to appear only on the north side of ridges. Extending the monitoring south of this area would also become more difficult from a coordination and crew availability perspective.

The decision to limit the sampling to stands of 10 to 20 years of age is because below 10 years of age, the disease may not be well expressed, and over 20 years of age, sampling becomes progressively more difficult because of increased tree size.



There are two strata in the sample design; 20 stands in 5 townships known to have SNC, and 40 stands in the rest of the western Oregon Cascades north of Oakridge. Sampled stands are within ownerships of SNCC members. SNCC members provided stand mapping of candidate stands and information on stand size, age, and past cultural treatments.

The dual strata-sampling design was done primarily for funding-related reasons. Our BLM office was prepared to fund SNC monitoring in the 20-stand localized area on lands within the grid of it's administered

lands. Beyond that, we did not have the resources to establish and monitor SNC levels and required assistance in funding and monitoring crews from outside sources.

There are 123 townships in the sampled area; over 2 million acres. The west border are townships where SNCC owners have substantial land holdings; we stopped when ownership became less than about a section per township because of the low probability of finding suitable candidate stands. The high elevation mountain hemlock-dominated townships form the east border, because we think they are unlikely to exhibit SNC symptoms.

This monitoring is being done with support of SNCC and ODF in conjunction with the aerial surveys done by ODF.

What has been accomplished

Training of all Cascade crews was done by Alan Kanaskie on March 20, 2001. This was done in the Oregon Coast Range at the Hebo Ranger Station and in the vicinity of Beaver.

During the week of April 9th Mike McWilliams provided one-on-one training with all Cascade crews. The crews were from Salem BLM, Mt Hood NF, Willamette NF, and Eugene BLM. Work started the following week and ended about mid-May. The main hindrance was snow that blocked access to National Forest lands.

One complication was the number of stands at upper elevations that were exposed to winds and ice storms. These stands may look like those severely affected with Swiss needle cast and have poor needle retention; however, there may be few if any pseudothecia.

Insect defoliators were found in our sampling. On Salem BLM lands, several stands had trees infested with silver-spotted tiger moth. Infested trees had 1 or 2 south-facing branches with no needles and with webbing. One of our Salem crew members, Darrell Foster, brought back a live caterpillar that was sent to Bruce Hostetler at the Forest Service's Westside Technical Center for positive identification. On the North end of the Willamette NF, many stands were infested with Cooley spruce-gall adelgids.

Some stands had laminated or Armillaria root rot. In root diseased areas, we attempted to locate plots outside of infected areas. Some of these undoubtedly are or will become infected, in which case new trees will be selected. We are looking at representative stand conditions rather than following symptoms in each tree.

There has been some coordination problems with the many crews that we used. Even with identical training, there were minor departures in how measurements were done. Ideally, it would be best to have two crews doing all of the work rather than four separate crews. Because of workloads, this was not possible.

Analysis is not completed. All information has been input into Excel spreadsheets similar to what Alan Kanaskie has done. I was able to get only 37 of the 40 stands I desired in the Extensive strata.

Preliminary analysis of Extensive strata

Of the two strata, thirty-seven stands had data collected with the following stand summaries:

Color 1-4 SNC rating 1-6

C1 SNC rating 1 10 stands

Color 1; Normal green color

SNC rating 1; Healthy normal-appearing Douglas-fir stand, 3.5 years of needle retention at mid crown

C1 SNC rating 2 4 stands

Color 1; Normal green color

SNC rating 2; Almost normal but showing slight yellowing, 3.5 years of needle retention at mid crown

C2 SNC rating 1 3 stands

Color 2; Slight yellowing

SNC rating 1; Healthy normal-appearing Douglas-fir stand, 3.5 years of needle retention at mid crown

C2 SNC rating 2 10 stands

Color 2; Slight yellowing

SNC rating 2; Almost normal but showing slight yellowing, 3.5 years of needle retention at mid crown

C2 SNC rating 3 7 stands

Color 2; Slight yellowing

SNC rating 3;Yellowing obvious, most trees retaining 2.5 to 3 years of needles

C2 SNC rating 4 1 stand

Color 2; Slight yellowing

SNC rating 4; Most trees retaining 1.5 to 2 years of needles, some reductions in height growth.

C3 SNC rating 2 2 stands

Color 3; Moderate yellowing

SNC rating 2; Almost normal but showing slight yellowing, 3.5 years of needle retention at mid crown

Preliminary analysis of Intensive Strata of 20 stands in 5 townships; results are as follows:

C1 SNC rating 1 2 stands

Color 1; Normal green color

SNC rating 1; Healthy normal-appearing Douglas-fir stand, 3.5 years of needle retention at mid crown

C1 SNC rating 2 3 stands

Color 1; Normal green color

SNC rating 2; Almost normal but showing slight yellowing, 3.5 years of needle retention at mid crown

C2 SNC rating 1 0 stands

Color 2; Slight yellowing

SNC rating 1; Healthy normal-appearing Douglas-fir stand, 3.5 years of needle retention at mid crown

C2 SNC rating 2 6 stands

Color 2; Slight yellowing

SNC rating 2; Almost normal but showing slight yellowing, 3.5 years of needle retention at mid crown

C2 SNC rating 3 10 stands

Color 2; Slight yellowing

SNC rating 3;Yellowing obvious, most trees retaining 2.5 to 3 years of needles

C2 SNC rating 4 0 stands

Color 2; Slight yellowing

SNC rating 4; Most trees retaining 1.5 to 2 years of needles, some reductions in height growth.

C3 SNC rating 2 0 stands

Color 3; Moderate yellowing

SNC rating 2; Almost normal but showing slight yellowing, 3.5 years of needle retention at mid crown

C3 SNC rating 3 1 stand

Color 3; Moderate yellowing

SNC rating 3;Yellowing obvious, most trees retaining 2.5 to 3 years of needles

About 1/4 of the stands in the extensive strata have normal color and no SNC symptoms whereas only 1/10 of the intensive strata stands meet the least amount of SNC criteria.

Douglas-fir is traditionally thought to have needle retentions of 5 to 8 years. I have personally seen stands of coastal Douglas-fir in the northeast side of Mount Hood where there are up to 8 years of needles. In our samples we have 36 of 370 trees that have 5 or more years of needles in the extensive strata. Most stands have about a 3-year complement of needles.

We do not know how SNC will develop in the Cascades stands that are now showing symptoms within the local areas, whether other stands within the affected area will begin showing symptoms, or if it will spread into adjoining areas. If within 5 years, SNC in the Cascades subsides, then we will have a documented history of this episode that can be consulted in case of future episodes. If the SNC infestation continues to develop, then we will be better able to prepare management strategies.

Plans for Next year

We plan on remeasuring the transects next spring. We will get training from ODF again for consistency. We hope to get the same crew people if possible.

Will Littke offered to provide pseudothecia counting on 200 trees; 5 trees in each of the 40 stands in the extensive strata. This would validate the presence of SNC in our samples and provide some measure of quantitative amounts of infection. I consider our attempts poor at best to determine SNC presence in addition to the symptoms. These collections would be from north and south sides of the middle 1/3 of the crown. Branchlets would be sealed into ziplock bags and stored in coolers, placed in refrigerators at the end of the day, and mailed weekly. Some type of pole pruning saw or clippers would be necessary for tall trees. Samples could also be sent to Lori Winton for her studies of the genetics of SNC. According to Will Littke, needles damaged by adelgids are not used.

The other problem is weather exposure and low fertility sites causing needle loss and yellowing. Also, we had originally thought to get understory vegetation, because use of the Plant Association Group modeling might be easier and more reliable.

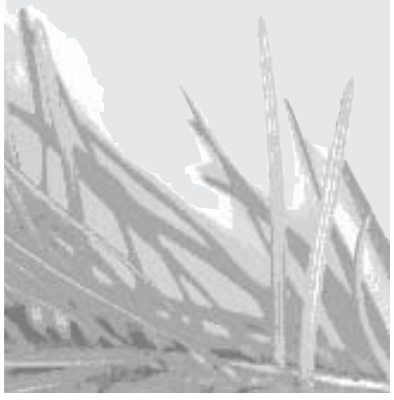
Growth Impact Study: Growth trends in the first 2-yr period following establishment of Phase III Permanent Plots and Pre-commercial Thinning Controls

Doug Maguire and Alan Kanaskie

Some additional analyses have been completed on data representing the first 2-yr growth period on Growth Impact Study (GIS) permanent plots. The objectives of the study were: 1) to monitor Swiss needle cast (SNC) symptoms and tree growth in 10-30-yr-old Douglas-fir plantations in north coastal Oregon; and 2) to provide an improved estimate of growth losses associated with a given initial level of SNC. Reported here are two major aspects of the study: 1) growth losses associated with differing intensities of SNC as measured by foliage retention (FOLRET) and crown length to sapwood ratio (CL:SA); 2) relation between growth and both soil and foliage chemistry on a subset of 15 GIS and 10 PCT (precommercial thinning study) plots.

Methods

In the late winter/early spring of 1998, a network of 76 permanent plots was established across locations previously sampled in Phases I and II of the Growth Impact Study. The plots were square and 0.08-ha (1/5-ac) in area (31.8 x 31.8 m). Each plot was centered on the 5th point of the ODF transect established in Spring 1997 (Phase I plots were centered on the 3rd point). On each measurement plot, all trees were tagged at breast height and a subsample of at least 40 Douglas-fir was measured for total height, height to crown base, and dbh at time of plot establishment. After two growing seasons, all trees were remeasured for dbh, and all trees from the original height subsample were remeasured for total height and height to crown base. Trees on each plot were also scored for SNC at time of plot establishment in 1998 and just prior to bud break in 1999 and 2000. On 10 dominant or codominant trees per plot, the crown was divided vertically into thirds, and the average number of years that foliage was retained in each third was estimated visually to the nearest 0.1 year. Plot ratings were computed as the average of all crown thirds from all ten trees. These same trees were cored to determine sapwood width and sapwood area at breast height. Sapwood area at crown base was estimated from sapwood area at breast height using a previously developed sapwood taper equation for Douglas-fir (Maguire and Batista 1996).



Methods

Field and Lab Work

At the same time that GIS plots were established in early 1998, 22 sets of PCT plots were also established across a range in initial SNC severity. The objective of the PCT study was to test whether thinning caused a change (increase or decrease) in SNC severity and associated growth losses. All installations or sets of plots included one control plot and one or two thinned plots. For the purpose of this analysis, only the control plots were considered for sampling. All tree tagging, initial tree measurements, SNC ratings, and tree re-measurement in 2000 were completed following the same protocol as applied in the GIS plots.

A subset of 25 permanent plots from two studies (GIS and PCT) was selected to represent the two disease extremes, specifically, severe SNC and little to no SNC. Ten PCT plots and 15 GIS plots were selected based on the 1997 foliage retention ratings. A composite soil sample from each plot was collected during tree remeasurement in late winter/early spring of 2000. Selection of soil and foliage samples was linked to five randomly selected SNC-rating trees per plot. Exact location of soil samples was determined by selecting a random azimuth from the tree and a random distance between 0 and 5 m from the tree. The duff and loose organic matter were lightly scraped away from the sample point, and a cylindrical core of mineral soil was extracted from the top 10 cm with a standard soil probe having a diameter of 2 cm. A second soil core was removed at the same distance from the tree but on the opposite side. The ten soil cores from a plot were placed together in the same plastic bag to produce a composite sample that was placed in a cooler and transported to the lab.

Foliage samples were collected from the same five randomly selected SNC-rating trees. The target branch for foliage sampling was the south-most branch in the fifth whorl from the top of the tree. All 1999 shoots arising from lateral buds on the terminal shoot were removed. Shoots from all trees were placed in a single plastic bag, and the composite sample was transported to the lab in a cooler. Due to the top height of older plots, foliage was sampled slightly lower than the fifth whorl on some trees.

Soil samples were analyzed for all macronutrients except iron, and foliage samples were analyzed for all macronutrients and some micronutrients. A fresh subsample was also reserved for assessment of fungal mass by the PCR (polymerase chain reaction) in Jeff Stone's lab.

Statistical Analysis

In the initial analysis of growth for the first 2-yr period, all variation in 1998 needle retention was assumed totally controlled by SNC intensity. Individual tree values were averaged for the 10 sample trees on each plot to arrive at a plot average (FOLRET98). A second SNC variable was computed as an index of crown sparseness, specifically, the ratio of crown length to sapwood area at crown base, abbreviated at CL:SA (Maguire and Kanaskie, in press). A simple growth model was then fitted to the data from all 76 GIS plots, with FOLRET98 and CL:SA as two possible predictor variables:

[1] $\ln[PAI] =$

$$b_0 + b_1 \bullet X_1 + b_2 \bullet X_2 + \ldots + b_k \bullet X_j + b_{k+1} \bullet FOLRET98 + b_{k+2} \bullet CL:SA$$

where PAI=plot-level periodic annual cubic volume growth of Douglas-fir and X_i=plot-level predictor variables.

Similar models were explored for estimating growth as a function of soil and foliage elements, but only for the 25 GIS and PCT plots from which soil and foliage were sampled.

Results and Discussion

Swiss Needle Cast Severity

Approximately 91% of the variation in cubic volume PAI was explained by the following model:

[1] $\ln[PAI] = -0.09601 + 0.8082 \bullet \ln(BA_{DF}) - 0.2081 \bullet \ln(BA_{total}) + 0.02458 \bullet SIB + 0.6914 \bullet \ln(FOLRET98) - 0.3052 \bullet \ln(CL:SA)$

where PAI = plot-level periodic annual cubic volume growth of Douglas-fir (m^3/ha)

BA_{DF} = initial Douglas-fir basal area (m^2/ha in 1998)

BA_{total} = initial plot basal area (m^2/ha in 1998)

SIB = Bruce's (1981) site index based on initial (1998) conditions (m at 50 yrs)

FOLRET98 = initial (1998) average foliage retention for plot

CL:SA = average CL:SA of dominant/codominant trees in 1998d

All variables were significant ($p<0.05$), although the parameter representing the intercept was not significantly different from zero ($p=0.76$). As expected, Douglas-fir growing stock was the major predictor, and alone it accounted for approximately 76% of the Douglas-fir volume growth; however, growth of the plots also increased as foliage retention increased and decreased as crown sparseness increased (Fig. 1). Assuming the healthiest stands were represented by the greatest value of FOLRET98 (3.1 yrs) and lowest value of CL:SA (2.74), the model implies the volume growth losses depicted in Fig. 2. The most severely impacted plot had FOLRET98=0.75 and CL:SA=11.1. These SNC conditions imply an average growth rate of 7.8 m^3/ha/yr (111 ft^3/ac/yr) vs. an expected value under optimal levels of FOLRET98 and CL:SA amounting to 31.5 m^3/ha/yr (451 ft^3/ac/yr). The inferred cubic volume growth loss for stands experiencing these conditions is therefore approximately 75%.

Although distinction between the biological implications of FOLRET98 and CL:SA are not clear, several differences are apparent. First, foliage retention is clearly related to needle longevity, whereas CL:SA pertains more directly to the density of foliage within the crown. Furthermore, neither is a direct measure of total foliage mass or area, although SA alone is presumed to be proportional to total leaf area of a tree. It may be that CL:SA reflects the cumulative effects of SNC and associated premature needle loss over a prolonged period, whereas FOLRET98 describes the condition for 1998 only. However, the two indices of SNC are related, as indicated by their correlation of 0.53.

Soil and Foliar Chemistry

Approximately 72% of the variation in Douglas-fir PAI was accounted for by differences among the plots in initial Douglas-fir basal area. This degree of explanatory power was similar to that exhibited for the 76 GIS plots. Two additional variables brought the R^2 up to 91%, which appeared to be the maximum regardless

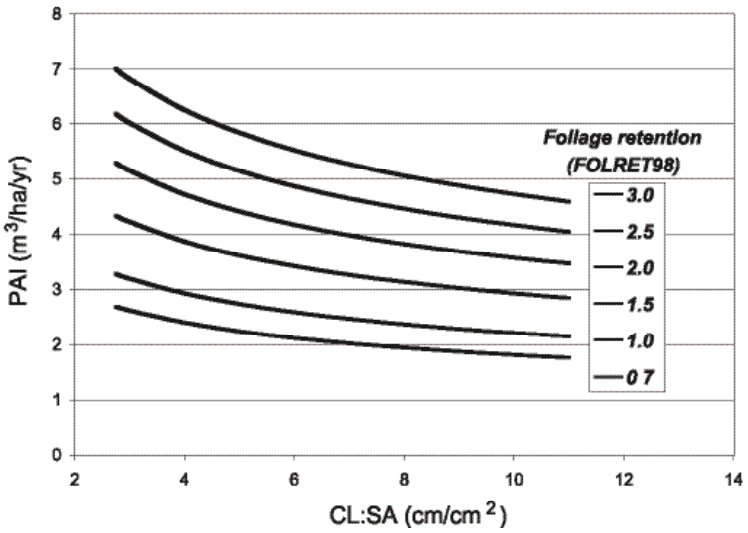


Figure 1. Relationship between cubic volume PAI and SNC severity as measured by both foliage retention (FOLRET98) and crown sparseness (CL:SA), assuming BA_{DF}=14.4 m^2/ha and BA_{total}=18.1 m^2/ha.

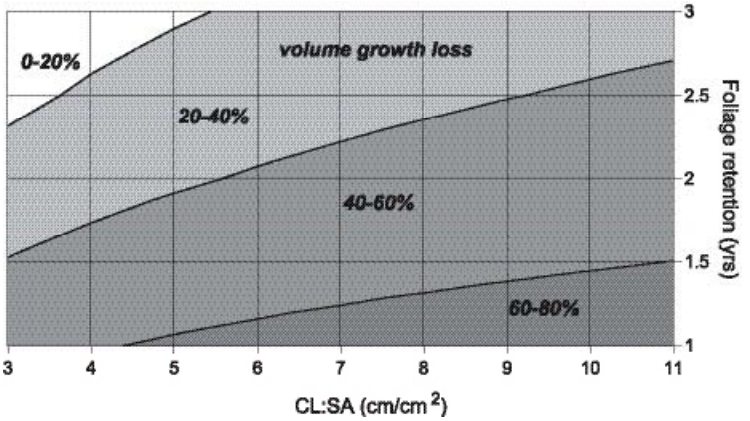


Figure 2. Volume growth losses associated with varying levels of foliage retention (FOLRET98) and crown sparseness (CL:SA), assuming BA_{DF}=14.4 m^2/ha and BA_{total}=18.1 m^2/ha.

of type and number of variables. The following model illustrates the form of the relationships between PAI and these two variables:

[1] $\ln[PAI] = 8.29423 + 0.72347 \bullet \ln(BA_{DF}) - 6.6853 \bullet N\% + 7.29891 \bullet \ln(N\%) - 1.99764 \bullet FOLRET98 + 4.62688 \bullet \ln(FOLRET98)$

where PAI = plot-level periodic annual cubic volume growth of Douglas-fir (m^3/ha)

BA_{DF} = initial Douglas-fir basal area (m^2/ha in 1998)

N% = foliar nitrogen content (% dry weight)

FOLRET98 = initial (1998) average foliage retention for plot

The response of PAI to both foliar nitrogen and foliage retention is curvilinear, with a peak at approximately 1.1% foliar nitrogen and 2.5 yrs foliage retention (Fig. 3). The initial increase in growth with increasing foliar nitrogen may not be too surprising since in other contexts increasing foliar nitrogen is generally associated with better growth. However, in north coastal Douglas-fir plantations, the highest levels of foliar nitrogen continue to be associated with poorer foliage retention as well as poorer growth. Two plausible mechanisms leading to this association are: 1) nutrient imbalances caused by a relative excess of nitrogen, in turn enhancing substrate for the causal fungus; or 2) concentration of nitrogen in surviving foliage caused by translocation from prematurely abscising needles and/or accelerated mineralization of nitrogen in the forest floor due to needle fall and greater light, temperature and moisture at ground level.

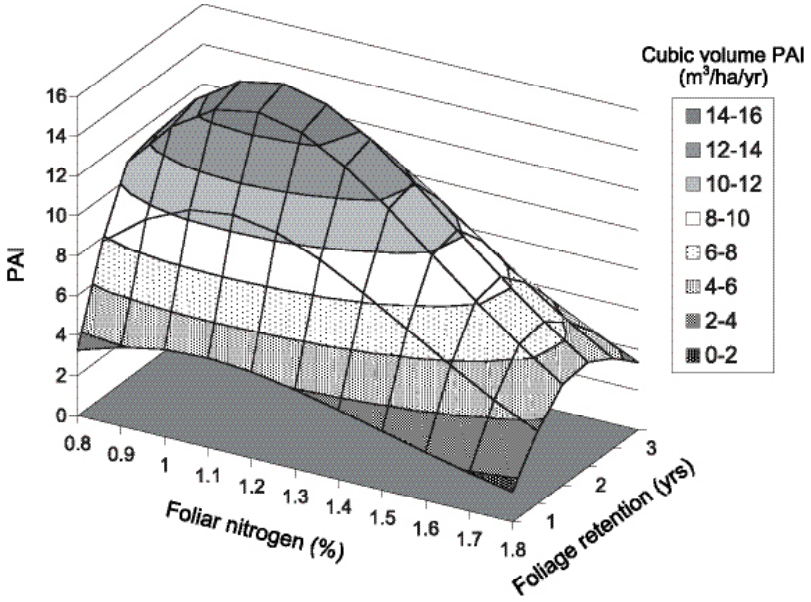


Figure 3. Cubic volume PAI in response to foliar nitrogen concentration and foliage retention, assuming an initial Douglas-fir basal area of 13 m^2/ha.

Literature Cited

Maguire, D.A., and Bastista, J.L.F. 1996. Sapwood taper models and implied sapwood volume and foliage profiles for coastal Douglas-fir. Can. J. For. Res. 26: 849-863.

Maguire, D.A. and A. Kanaskie. The ratio of live crown length to sapwood area as a measure of crown sparseness. Forest Science, *in press*.

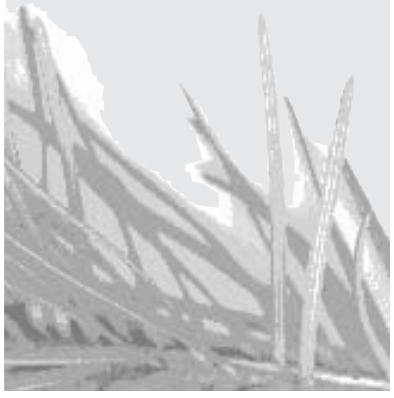
Response of Swiss needle cast severity and tree growth to pre-commercial thinning in north coastal Oregon

Alan Kanaskie and Doug Maguire

Possible interactions between thinning and Swiss needle cast (SNC) severity are being monitored in the Pre-commercial Thinning (PCT) Study. Reports continue to be received about stands declining immediately after thinning, even though they appeared to have no significant levels of SNC prior to thinning. In areas free of noticeable SNC, thinning has frequently been implemented apparently without promoting SNC. The objectives of the PCT study were: 1) to test the effect of thinning on SNC severity; and 2) to test the effect of thinning on growth responses under varying levels of initial SNC severity; and 3) to test for interactive effects of initial SNC severity and thinning intensity on subsequent SNC severity and growth.

Methods

In the later winter/early spring of 1998, 22 sets of plots were established across a range in initial Swiss needle cast (SNC) severity. Most of these sets contained a pair of plots, one thinned and the other a control, but some included a third plot that was thinned to a lower residual density. The thinning prescription specifically called for leaving 494 tph (200 tpa), but on two installations the target residual was 247 tph (100 tpa). In addition, 5 installations with a 494-tph plot were given a third plot to be thinned to 247 tph. All control plots and 494-tph plots were square and covered 0.08-ha (1/5-ac; 31.8 x 31.8 m), except for the two control plots whose only corresponding thinned plot had a residual of 247-tph. These latter two control plots and all 7 plots thinned to 247 tph encompassed an area of 0.16-ha (2/5-ac). On each measurement plot, all trees were tagged and measured for diameter at breast height. At least 40 Douglas-fir trees on each plot were also measured for total height and height to crown base. The treated plots were thinned before the growing season started in 1998. After two growing seasons (1998 and 1999), all trees were remeasured for dbh, and all trees from the original height subsample were remeasured for total height and height to crown base. Where necessary, replacement trees for the height subsample were substituted with another tree of the same diameter. Ten dominant or codominant trees on each plot were also scored for SNC at time of plot establishment in 1998 and during annual visits in the spring of 1999 and 2000.



The change in SNC severity from 1998 to 2000 was statistically analyzed in three ways: 1) assuming a randomized block design with no

covariates but plot pairs treated as blocks and plots classified as thinned or control (same as a paired t-test); 2) as a multiple regression problem with a large number of potential predictors such as initial stand density, thinning intensity, and initial SNC severity; and 3) under a randomized block design with a large number of potential covariates (analyzed with a multiple regression model).

Growth analysis for the first 2-yr period was likewise accomplished by successive regression models that accounted for the blocking structure both by discrete block effects and by covariates that recognized differences in initial conditions and thinning intensities among both blocks and plots.

Results and Discussion

Swiss needle cast severity

Changes in SNC severity had no significant relationship to thinning during the first 2-yr growth period after thinning. However, significant effects of initial foliage retention (RET98) and the ratio of initial crown length to sapwood area (CL:SA) were identified, with stand age appearing as an additional factor. The final regression model was as follows:

[1] $dRET = -1.55223 + 1.928 \bullet RET98 - 3.82587 \bullet \ln(RET98) - 2.57642 \bullet CL:SA + 7.90351 \bullet \ln(CL:SA) - 0.05364 \bullet AGE98$

where dRET = change in foliage retention from 1998 to 2000
RET98 = average plot foliage retention in 1998
CL:SA = average crown length to sapwood area ratio in 1998
AGE98 = average plot breast height age in 1998

This model explained 72% of the variation in change in needle retention, with all variables significant ($p<0.01$). The corresponding response surface with respect to RET98 and CL:SA is shown in Fig. 1. Results suggested that stands starting with a moderate level of foliage retention changed little over the past two years, while those starting with high or low levels of foliage retention improved by retaining foliage up to 1 yr longer. In contrast, stands with intermediate levels of crown sparseness (CL:SA) experienced the greatest improvement in needle retention, although relatively sparse and relatively dense crowns experienced some decline in retention, but the maximum decline was only 0.5 yr.

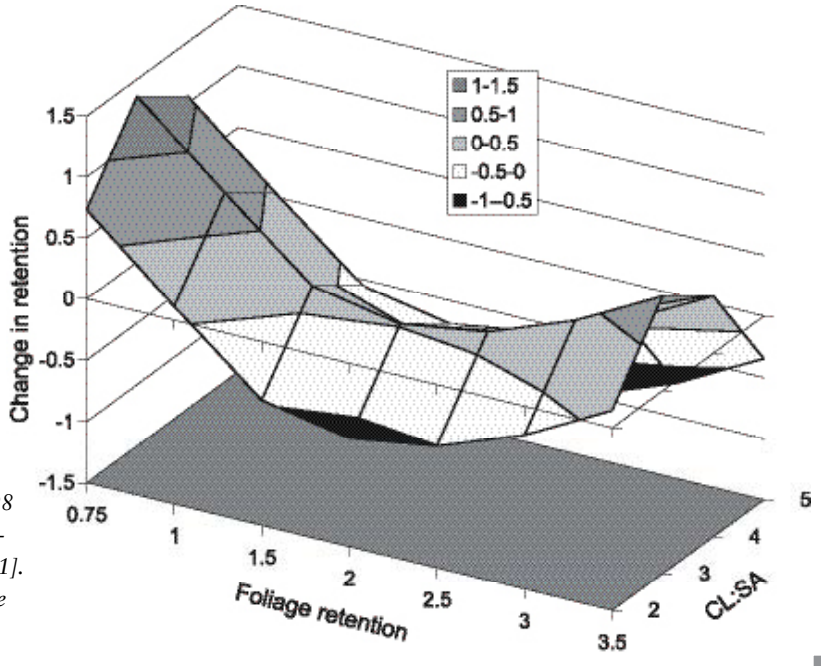


Figure 1. Change in needle retention between 1998 and 2000 for thinned and control plots in the pre-commercial thinning study, as predicted by model [1]. Stand breast-height age is fixed at the plot average of 11 yrs.

Plot growth in response to thinning

A large portion of the variation in plot growth (72%) was accounted for by the blocking structure imposed by the sets of plots. However, a lot of the block effect could be explained by differing plot conditions, exemplified in the following model:

[2] $\ln(VPAI) = -3.62974 + 1.00999 \bullet \ln(BA_{DF}) - 0.06922 \bullet BA_{RA} - 1.40278 \bullet BA_{OC} +$

$$4.5374 \bullet RD_{OC} - 0.01936 \bullet resBA_{TOT} + 0.97410 \bullet \ln(SIB98) + 0.17982 \bullet RET98 + 0.13960 \bullet dRET98$$

where VPAI = Cubic volume periodic annual increment for plot (m^3/ha/yr)

BA_{DF} = initial Douglas-fir basal area (m^2/ha)

BA_{RA} = initial red alder basal area (m^2/ha)

BA_{OC} = initial basal area in other conifers (m^2/ha)

RD_{RA} = initial relative density (Curtis 1982) of other conifers

$resBA_{TOT}$ = total residual basal area (m^2/ha)

SIB98 = site index (m at 50 yrs)

RET98 = average plot foliage retention in 1998

dRET98 = change in RET98 from 1998 to 2000

This model accounted for 94% of the variation in cubic volume PAI, and parameters for all variables were significantly different from zero (all $p<0.01$). The model implied that volume growth was largely a function of standard variables such as initial Douglas-fir growing stock (BA_{DF}), competitive effects of red alder and other conifers before thinning (BA_{RA} and BA_{OC}), residual basal area of competitors ($resBA_{TOT}$), and site index (SIB98). Although SNC severity itself did not readily enter into the model, when combined with the change in foliage retention over the 2-yr growth period, foliage retention did become a significant covariate. As would be expected, a higher initial foliage retention implied a greater volume growth. Likewise, an increase in foliage retention over the 2-yr period, for a given initial retention, was associated with greater growth (Fig. 2).

A more explicit test of the interaction between thinning intensity and initial SNC condition was accomplished by fitting the following model:

[3] $$\ln(VPAI) = -2.75102 + 1.01323\ln(BA_{DF}) - 0.02166 \bullet resBA_{TOT} - 0.03391 \bullet cutBA_{TOT} + 0.75982 \bullet \ln(SIB98) + 0.12267 \bullet RET98 + 0.01212 \bullet cutBA_{TOT} \bullet RET98 + 0.19097 \bullet dRET98$$

where VPAI, BA_{DF}, $resBA_{TOT}$, SIB98, RET98, and dRET98 are as above and

$cutBA_{TOT}$ = total cut basal area (m^2/ha)

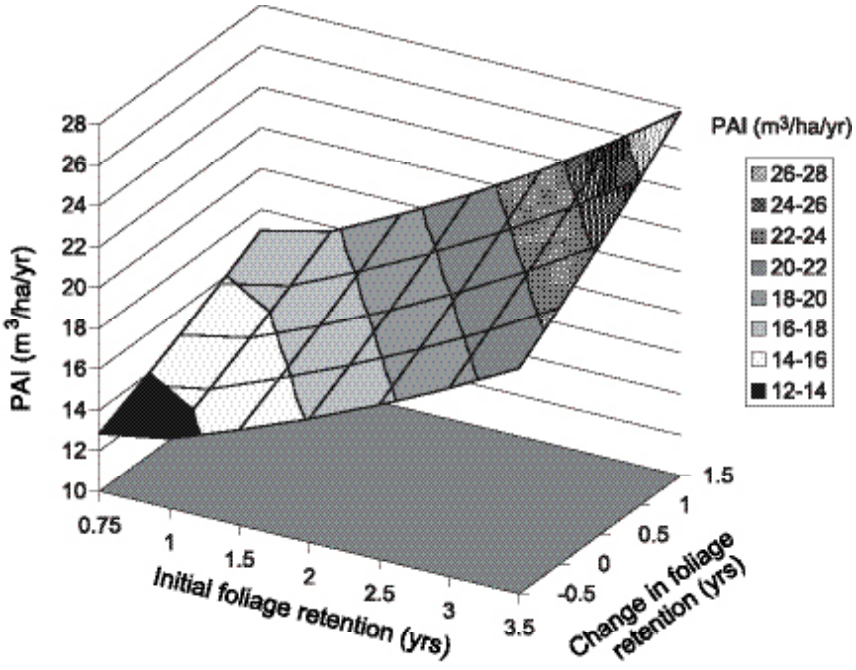


Figure 2. Volume PAI under varying levels of initial foliage retention (RET98) and differing levels of change in RET98 (dRET98), as predicted by model [2].

This model accounted for 91% of the variation in cubic volume PAI and, with the exception of RET98, all parameter estimates were significantly different from zero ($p<0.01$). The p-value for RET98 was 0.07, indicating borderline significance. The effects of the stand structural variables were consistent with model [2], and the negative effect of total cut basal area was probably attributable to the fact that greater cut basal area for a given residual basal indicates a greater initial density and associated reduced growth potential of individual trees (Fig. 3). However, the interaction of cut basal area and foliage retention also indicated that the effect of a given level of foliage retention was greater with at a greater level of cut basal area, all else being equal. Alternatively, the negative effect of cut basal area is reduced at a higher level of initial foliage retention (Fig. 4).

The range in cubic volume growth loss implied by the maximum foliage retention of 3.4 yrs and minimum of 0.7 yrs was 38% for model [2] and 28% for model [3], assuming no change in foliage retention and no thinning, respectively. Under the average thinning intensity of 6 m^2/ha, the corresponding growth loss estimated from model [3] was 41%. Although these results suggested the possibility that thinning exacerbated the effect of SNC on growth, additional analysis is being conducted on the first growth period, and the 4-yr remeasurements will be available soon.

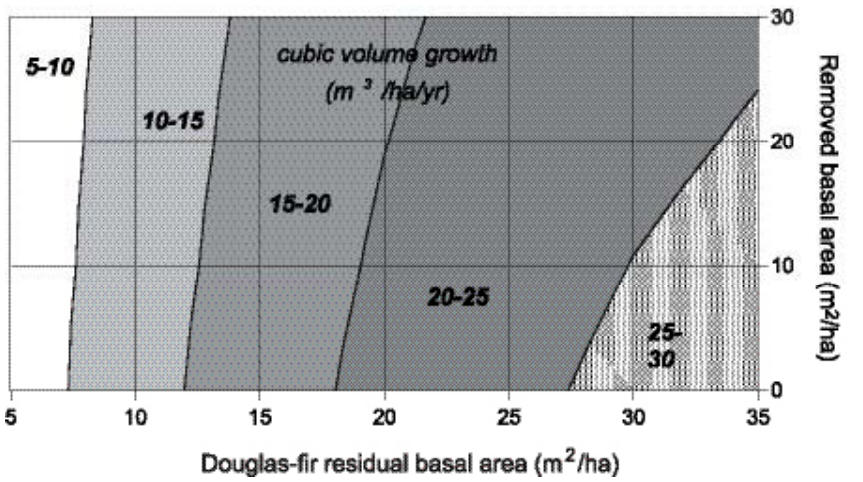


Figure 3. Volume PAI assuming pure Douglas-fir stands ($BA_{DF}=BA_{total}$), and varying intensities of thinning ($cutBA_{TOT}$), as predicted by model [3]. Foliage retention was assumed to be 2.5 yrs, site index was assumed to be 44 m (50 yrs), and dRET98 was 0.16.

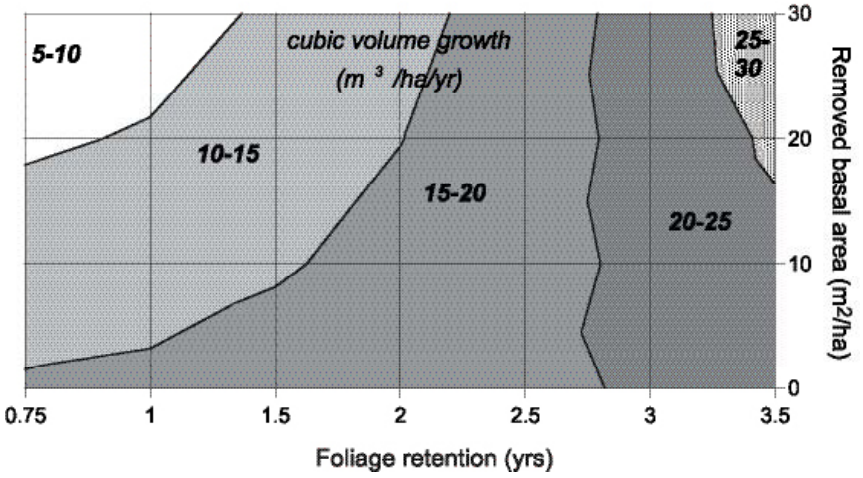


Figure 4. Volume PAI under varying levels of initial foliage retention (RET98) and differing cut intensities ($cutBA_{TOT}$) as predicted by model [3]. Other covariates were assumed to be $BA_{DF}=16.8$, $resBA_{TOT}=16.8$, SIB98=44, and dRET98=0.16.

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Maguire, D.A., and Bastista, J.L.F. 1996. Sapwood taper models and implied sapwood volume and foliage profiles for coastal Douglas-fir. Can. J. For. Res. 26: 849-863.

Maguire, D.A. and A. Kanaskie. The ratio of live crown length to sapwood area as a measure of crown sparseness. Forest Science, *in press*.

Population Structure of Phaeocryptopus gaeumannii

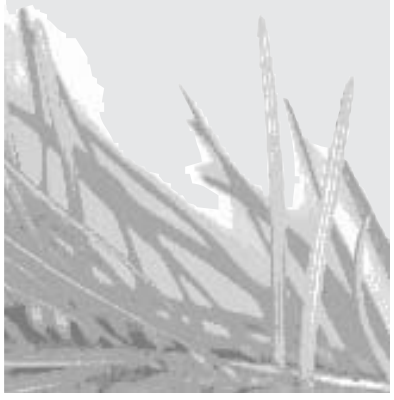
Loretta M. Winton, Everett M. Hansen, and Jeffrey K. Stone

Abstract

A survey of the genetic diversity and population structure of the Douglas-fir Swiss needle cast pathogen *Phaeocryptopus gaeumannii* was conducted using single-strand conformational polymorphisms (SSCP) to screen for allelic variability in mitochondrial and nuclear housekeeping genes. Thirty populations were sampled both within the natural range of Douglas-fir and where the tree was planted as an exotic. DNA sequences of SSCP alleles were used to construct multilocus gene genealogies and to test various hypotheses of recombination (outcrossing) and clonality (selfing). We found that *P. gaeumannii* in the region of Oregon's Swiss needle cast epidemic is subdivided into two reproductively isolated sympatric lineages. Low genetic diversity with the presence of overrepresented genotypes in both lineages suggests a predominantly selfing reproductive mode. One lineage has nearly worldwide distribution, occurring throughout much of the Pacific Northwest as well as in exotic locations that have historical reports of disease. The second lineage is restricted to Oregon's coastal region. There was suggestive evidence that disease severity was positively correlated with the abundance of this second lineage in young plantations in the epidemic area.

Introduction

Although the hypothesis of a novel *P. gaeumannii* strain has often been postulated it has never been tested, and nothing is known about fundamental life history traits of the fungus such as its genetic structure or geographic differentiation. However, such information is essential for identifying the source of the Tillamook epidemic and developing effective management strategies to control it. The tools of population genetics and molecular and evolutionary biology have recently demonstrated the power that explicit tests of reproductive mode can have on understanding the diverse life histories of fungal pathogens (for a recent review see Taylor et al 1999). The emerging theme is that reproductive mode (selfing or outcrossing) is uncoupled from reproductive morphology (sexual or asexual) to the extent that virtually all fungi, including those with no known means of sexual reproduction, exhibit both clonal and recombining population structures in nature. Therefore, in this report, "reproduction by recombination is defined as the production of progeny genomes that are mixtures of genetically different parental genomes, and reproduction by clonality is defined as the production of progeny genomes that are



identical to the parental genome" (Taylor et al 1999).

In this vein, we have utilized single-strand conformation polymorphism (SSCP; Orita et al 1989) to screen for DNA sequence variation at five loci in the *P. gaeumannii* genome. Hypotheses of clonality (selfing) and recombination (outcrossing) were evaluated with both population genetic and phylogenetic theory to test whether there is evidence of mixis throughout the entire range of the fungus or whether the species is geographically differentiated or reproductively isolated.

Material and Methods

Sampling and isolation. The locations of the 30 sampled populations and the number of individual ascospores sampled from each are presented in Table 1. Because ascospores are dispersed by wind and rain, a population was arbitrarily defined as a single plantation or stand of the Douglas-fir host. We sampled 17 populations within the main native range of Douglas-fir. Ten of these were within epidemic area of Oregon's Coast Range (between Waldport and Astoria within 18 miles of the coast) and four of the 10 were mature stands at least 80 years old. All other stands in the study were between the ages of 10 and 25 years. Seven of the native populations were located at a range of distances outside of the epidemic area. Because the fungus has previously caused disease only where Douglas-fir was planted as an exotic species, we also sampled from 13 locations outside of the contiguous Douglas-fir range. Single isolates were obtained from 5 to 21 trees per stand; a total of 402 isolates were tested.

Trees selected for sampling were non-adjacent and haphazardly chosen within a stand with no regard to apparent disease level. Branches with foliage bearing pseudothecia were collected, transported to the lab, and prepared for isolation. Single-ascospore *P. gaeumannii* individuals were obtained by adhering needles bearing only *P. gaeumannii* pseudothecia to lids of petri plates filled with water agar. Needles were arbitrarily selected from the most recent needle cohort bearing mature pseudo-thecia. Samples were incubated in a moist chamber for 3-5 days at 17 °C and one individual ascospore per host tree was removed from the agar surface and grown at 17 °C for 3-5 months on potato dextrose agar (Difco Laboratories, Detroit, MI).

DNA manipulations and data analysis. After DNA extraction, isolates were genotyped at the calmodulin (CAM), chitin synthase 1 (CHS), ± –tubulin (ATUB), ≤– tubulin (BTUB), and the mitochondrial ribosomal small subunit (mSSU) loci by means of single-strand conformation polymorphism (SSCP), a quick screening technique used to reveal DNA sequence variation. To ensure that putative alleles inferred from patterns on SSCP gels corresponded to unique nucleotide sequences, each allele was sequenced from at least three randomly chosen representatives.

Because *P. gaeumannii* is presumed haploid with a potentially mixed reproductive mode (selfing

Table 1. P. gaeumannii sampling locations

Region	Number [b]	Population	Location	N [d]
East U.S.	1	New Mexico	Lincoln National Forest	5
	2	New York	Lansing	12
	3	Vermont	Burlington	17
Overseas	4	England	Dunheld, Perthshire	15
	5	France	Epinal	14
	6	Germany	Grosshansdorf	14
	7	Italy	Tosi	17
	8	New Zealand North Island	Rotorua	16
	9	New Zealand South Island	Dunedin	21
	10	Switzerland1	Horgen	8
	11	Switzerland3	Rapperswil	8
	12	Switzerland4	Schaffhausen	5
	13	Switzerland5	Zurich	21
PNW[a]	14	Canby	Canby, OR	5
-nonepidemic	15	Foster Dam	Sweethome, OR	9
	16	Gold Beach	Gold Beach, OR	18
	17	MacDonald Forest	Corvallis, OR	16
	18	Olympia	Olympia, WA	16
	19	Phipps	Elkton, OR	11
	20	Toledo	Toledo, WA	17
PNW [a]	21	Bixby [c]	Beaver, OR	18
- epidemic	22	Coal [c]	Nahalem, OR	18
	23	Drift Creek	Waldport, OR	17
	24	Edwards [c]	Tillamook, OR	11
	25	Juno Hill	Tillamook, OR	16
	26	Limestone	Beaver, OR	14
	27	Lower Stone	Tillamook, OR	8
	28	North Fork	Nahalem, OR	11
	29	Prairie [c]	Tillamook, OR	15
	30	Upper Stone	Tillamook, OR	9

[a] Pacific Northwest. [b] Population identification number [c] Mature Stand > 80 years old. [d] Number of individual ascospores sampled.

and outcrossing), three multilocus permutation tests suitable for detecting recombination and population structure in haploid organisms were employed. All tests used the variable positions in DNA sequence data inferred from SSCP.

Results

Gene diversity and sequencing. Four alleles were detected at the BTUB locus and five at mSSU. ATUB, CHS, and CAM were each biallelic. There was no obvious geographic pattern to allelic distribution except that many loci tended to be fixed (monomorphic) in localities outside of the main contiguous native Douglas-fir range and especially where Douglas-fir is planted as an exotic. These results are consistent with the hypothesis of reduced genetic diversity in small populations due to founder events and genetic drift.

Of 160 theoretically possible multilocus genotypes, only 10 were found. Of these, two were found exclusively in the U.S. populations sampled outside of the contiguous natural Douglas-fir range (New Mexico, New York, and Vermont). Because these populations were small and geographically and environmentally isolated from the rest of the U.S. populations, we reasoned that they lacked opportunity to outcross with other genotypes. Therefore, these genotypes were excluded from tests of recombination. Of the remaining eight genotypes, three were widely distributed in overseas populations and those sampled in the Pacific Northwest. Five genotypes were found exclusively in the Pacific Northwest.

The two most common genotypes comprised 67% of the genotypes found in the entire data set and 85% of the genotypes found in the epidemic area. While the presence of overrepresented genotypes and association among alleles at different loci provide robust and significant evidence of clonal reproduction (Tibayrenc et al 1991), these features do not rule out recombination. That only two genotypes were overrepresented is suggestive of an "epidemic" type population structure, which can bias explicit tests for recombination towards clonality (Maynard Smith et al 1993). Therefore, the following tests for recombination were performed on a reduced data set that was "clone-corrected" by the removal of duplicated genotypes.

Population structure. We used the information on genotype distribution to assume that those present in the coastal fogbelt had the opportunity for recombination, whether or not they were capable of doing so. After adjusting for overrepresented genotypes (clone-correction), we used two methods to explicitly test for deviations from recombination (Figure 1). There was strong evidence that multilocus gametic disequilibrium (I_A) differed from that expected in a single, recombining population ($p = 0.003$, Figure 1a).

The phylogenetic approaches also supported deviation from

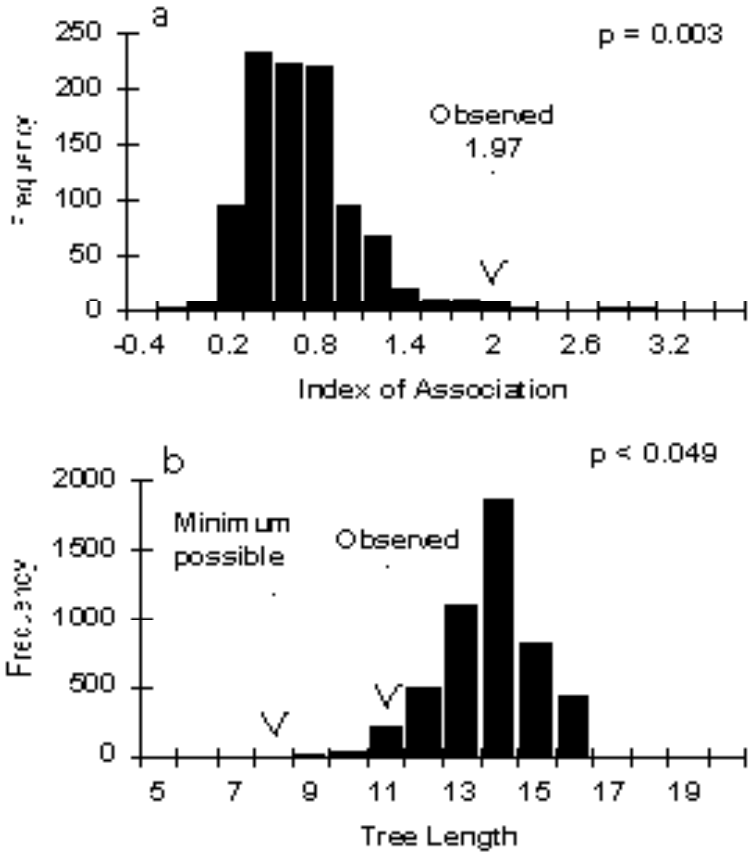


Figure 1. Comparison of the observed dataset to permutated datasets in which alleles have been randomly shuffled across isolates to simulate recombination (histograms). (a) Index of association permutation test. (b) Parsimony tree length permutation test.

complete panmixia. While separate genealogies for each of the five loci were well-resolved and of minimal length (i.e. no homoplasy), the four most parsimonious trees based on data from all four loci (a total of eight informative sites) indicated extensive incompatibility among genealogies from different loci and subdivided genotypes into two reproductively isolated groups (Figure 2). The most parsimonious trees from the combined genealogies were 11 steps in length, 3 longer than the minimum possible (1 step for each parsimony informative site = 8). Randomly shuffling the gene sequences among genotypes, leaving the linkage of nucleotides within loci intact, provided the null distribution for the recombination hypothesis. In 1000 such randomizations, 49 trees were found as short or shorter than the observed most parsimonious trees. This provided suggestive evidence against recombination, but it could not, conclusively, be ruled out (p = 0.049, Figure 1b). Whether the divergence of genotypes within lineages was the result of recombination or mutation could not be resolved with these data.

The partition-homogeneity test was used to test the null hypothesis of clonality. In this case, the sum of the individual locus tree lengths constructed from the observed data set (summed length = 8) was compared to summed tree lengths in randomized data sets, where nucleotide sites were randomly shuffled among loci. At the 95% confidence level, there was no evidence that trees constructed from the different loci were incompatible (p = 0.072, informative sites only, range of the summed tree lengths from randomized data was 8-11). Under these criteria the null hypothesis of clonal reproduction could not be rejected.

While geographic patterns of genotype distribution in the western United States were not obvious from the raw data set, mapping the two lineages as separate entities was revealing (Figure 3). Lineage 1 was the only lineage present in the four northern, interior populations and comprised the overwhelming majority (94%) of isolates from the MacDonald Forest stand in the eastern foothills of the Coast range. It was also present in varying quantities in all populations in the epidemic area (31% to 86%). Conversely, lineage 2 was the only lineage present at the Gold Beach site on the southern Oregon coast and comprised the majority of isolates (73%) from the site near the Oregon Department of Forestry D.L. Phipps Forest Nursery. The proportion of this lineage varied (14% to 69%) in sites within the epidemic area.

There were correlations between the proportions of the two lineages and Swiss needle cast disease severity measurements at five young planta-

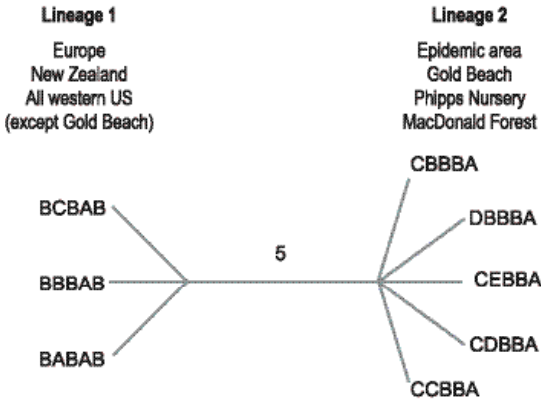


Figure 2. Genealogy constructed from informative nucleotide positions in the BTUB, mSSU, ATUB, CHS, and CAM loci, respectively, for genotypes present in native populations only. The geneaology collapsed onto a single branch subdividing genotypes into two reproductively isolated groups separated by five fixed nucleotide changes. All locations where these genotypes were found are also shown.

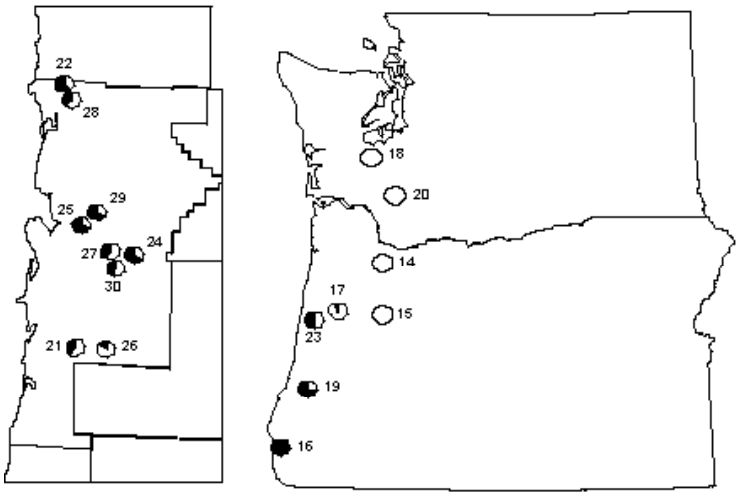


Figure 3. Relative proportions of lineages 1 (○) and 2 (●) and their distribution in Oregon and Washington Douglas-fir stands. Population numbers and locations are given in Table 1. Inset shows Tillamook county, the area with the greatest incidence and severity of Swiss needle cast (Hansen et al 2000).

tions within the epidemic area for which we had sufficient data (Figure 4). For example, canopy density, a measurement of needle casting, decreased significantly as lineage 2 contributed a greater proportion of individuals to the pathogen population of the stand ($P < 0.02$, Figure 4A). While the sample size was small, this implicates lineage 2 in the Tillamook epidemic. In addition, there was suggestive, but inconclusive, evidence that foliar discoloration increased with increasing proportions of this lineage ($p = 0.078$, Figure 4B).

Discussion

That *P. gaeumannii* sexually reproduces has never been in question; there is direct evidence wherever Douglas-fir is grown. Its ability for self-fertilization was demonstrated by Hood (1977). However, the role of recombination in the life history of the fungus has, prior to this report, never been addressed. In early phases of data acquisition and analysis it appeared that the Swiss needle cast outbreak in Oregon might simply be explained by high levels of genetic diversity and, by implication, recombination leading to genotypes more successful under current management or environmental conditions. However, the use of a phylogenetic approach for testing hypotheses on recombination and clonality revealed unexpected results that established a much more complicated picture.

We found that *P. gaeumannii* in Oregon is subdivided into two genetically differentiated groups that occur sympatrically in many coastal Douglas-fir stands, particularly within the region of the current Swiss needle cast outbreak. Multiple gene genealogies suggest that the two groups are reproductively isolated lineages that by the biological and phylogenetic species concepts constitute cryptic species. However, recent admixture of the two lineages in coastal forests, combined with a low outcrossing rate, might also explain the absence of recombined genotypes. Laboratory crosses could demonstrate whether the ability of the two lineages to mate has been lost, but only intensive, long-term monitoring would provide relevant evidence in natural circumstances.

One lineage (lineage 1) is widespread, occurring throughout much of the natural range of the pathogen, and its host, in Oregon and Washington. This was the sole lineage found in Europe and New Zealand, two regions with historical reports of disease. Most of the populations sampled in these areas harbored at least two, and usually all three, genotypes of the lineage. The presence of one of these genotypes in great excess within Oregon's epidemic area implies reproduction by clonal processes. We currently lack methodologies to estimate the relative amounts of both reproductive modes in nature, however they are currently being developed (Maynard Smith and Smith 1998). Because this lineage was the sole lineage found in four healthy stands at the northeastern limits of our sampling in the native Douglas-fir range, we tentatively propose that it is derived from there. The second lineage (lineage 2), comprised of five genotypes, was found only in western Oregon and in most of these stands was represented by only a single genotype. Maynard Smith et al (1993) refer to this as an epidemic population structure, distinguished by the occurrence of one of a few highly successful genotypes. By the same reasoning as described above, this lineage may be derived from the

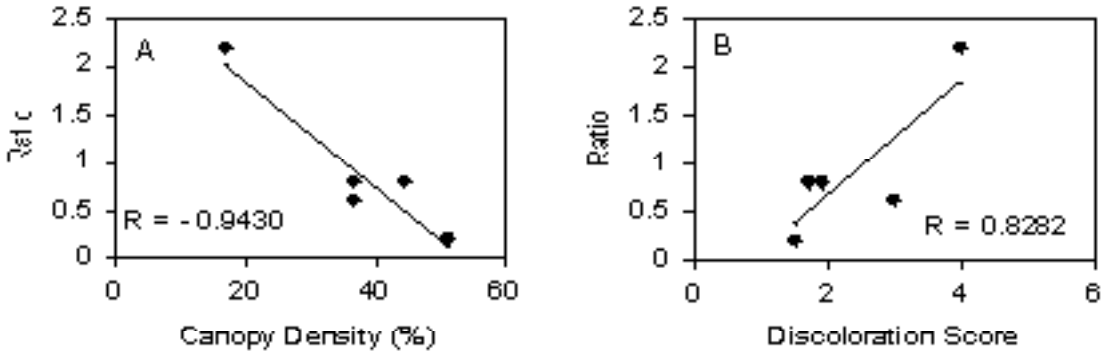


Figure 4. Pearson correlations between the ratio of the two reproductively isolated lineages (lineage 2 : lineage 1) and the symptoms canopy density (A) and foliar discoloration (B) at five young Douglas-fir plantations within the area of the Tillamook epidemic.

southern end of the Oregon coast, whence it has moved north through the coastal fogbelt. The next step is to confirm these proposed origins with an expanded latitudinal and longitudinal sampling strategy in the native range of Douglas-fir.

Both lineages were found, in varying frequencies, in the Swiss needle cast epidemic area between Waldport and Astoria, OR. Attempts to correlate the relative abundance of the two lineages with stand-level disease severity met with limited success and implicated lineage 2 in the Tillamook epidemic. As lineage 2, represented largely by a single genotype, increased in stands, canopy density was significantly reduced and there was slight evidence that foliage was more discolored. Compared to lineage 1, twice as many lineage 2 isolates were obtained from the most severely diseased sites and only about half as many from relatively healthy stands.

We demonstrated that the genetic diversity of the pathogen within Douglas-fir stands located in Oregon's epidemic is not homogeneous and all the stands we sampled in the area harbored genotypes from both lineages. The correlations we observed between symptoms of Swiss needle cast and a specific strain or lineage of *P. gaeumannii* was highly suggestive, but not, in our opinion, conclusive. The sample size we had available to make these comparisons was very small. In addition, disease ratings, notoriously difficult for this pathogen, were obtained at the stand- rather than tree-level. However, there is substantial variation in the symptoms of disease among infected trees (Hansen et al 2000) and it would be interesting to know how much of this variation can be explained by differences in the pathogenicity of the two lineages. Spore dispersal mechanisms and evidence of selfing, suggests that individual trees are likely to be infected by the same *P. gaeumannii* genotype. Future attempts at more detailed analyses of population structure seem crucial and should focus on the relative pathogenicity of the two lineages, their neighborhood sizes, and how these variables correlate with tree-to-tree variability in disease expression in the forest.

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Stomatal Regulation In SNC-Infected Foliage

Dan Manter and Katy Kavanagh

Background

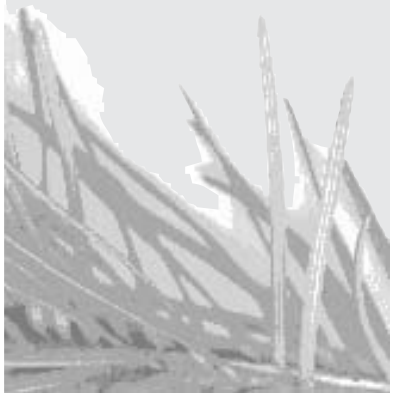
Stomata are the main entry points of CO_2 into actively photosynthesizing needles; therefore, the diurnal pattern of stomatal conductance (openness) will influence needle productivity. In a previous study, we showed that maximum rates of gas exchange (CO_2 and H_2O) are reduced by the presence of *Phaeocryptopus gaeumannii* fruiting bodies in needle stomata (Fig 1. from Manter et al. 1999). Theoretically, since this flux of water is reduced (i.e., water leaving needles), then more water should be available in *P. gaeumannii*-infected trees allowing stomata to stay open longer during the day. Finally, since needle water content is determined by the balance between water supply through xylem tissues and water lost through needle stomata, if SNC influences water supply this compensation may not be possible.

Objectives

Investigate the balance between water supply and water loss in Douglas-fir foliage infected with *P. gaeumannii* and its effect on patterns of stomatal regulation.

Methods

We investigated patterns of stomatal conductance (g_s) and water supply (K_{L_B}) from three sites with varying levels of SNC (Beaver, Hebo and Mac sites). Starting in June 1999 diurnal analysis of gas exchange (H_2O) and water potential were measured. Diurnal measurements included predawn water potentials, bi-hourly gas exchange (Li-1600) and leaf water potential (Ψ_{leaf}, a measure of water content) measurements commencing at dawn or immediately after foliage dried-off. At the end of the day, all measured foliage was removed and measured for leaf area, dry weight (used to express gas exchange on a unit basis) and fungal colonization.



Based on previous physiological work, proper stomatal regulation (control of needle water contents above a critical threshold) results in a linear relationship between g_s and the natural log of vapor pressure deficit (D) when lnD is 0-1 kPa (Oren et al. 1999) (Figure 1). In addition, this relationship can be described mathematically by equation 1:

$$g_s \bullet D = K_L \bullet (\Psi_{soil} - \Psi_{leaf}) \qquad (1)$$

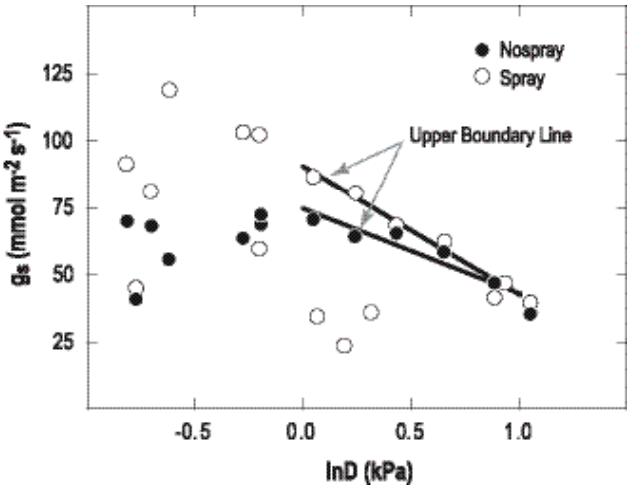


Figure 1. Response of g_s to lnD in one-year-old needles from spray and nospray trees (n = 3) at the Beaver-south plot. Data were collected on four different days in June and July 1999. The upper boundary line regression (0 ≤ lnD ≤ 1) was used to compute values in Table 1.

where g_s is stomatal conductance, D is the leaf-to-air vapor pressure gradient, K_L is whole plant leaf-specific conductance, Ψ_{soil} and Ψ_{leaf} are water potential of the soil and leaf, respectively. In other words, this equation means that the loss of water from needles (left side of the equation) is equal to the supply of water (right side).

Results & Discussion

The ability for xylem tissues to supply water was K_{L_B} was significantly lower in the nospray trees compared to sprayed trees (Beaver and Hebo sites only, see Figure 1). K_{L_B} is influenced by the ratio of leaf area to conducting sapwood area, as well as, the permeability of the sapwood. Changes in K_{L_B} could not be attributed to LA:SA ratios, since a single consistent relationship was observed. However, changes in stem permeability, assessed by the percent of latewood present, were significantly correlated with measured values of K_{L_B} (Figure 2), *i.e.*, as latewood increased, K_{L_B} declined. An inverse relationship between latewood presence and K_L is expected (Zimmerman, 1983) due to the comparatively smaller diameter of latewood cells as compared to earlywood cells (Megraw, 1986).

A theoretical analysis of equation 1 predicts that g_{sref} (g_s @ 1 kPa) is equal to $172.4 \bullet K_L$ (Oren et al. 1999). Using regression analysis, the best equation to explain our observed patterns of g_{sref} is $g_{sref} = 172.4 \bullet (0.009 \bullet K_{L\_B}) - 0.253 \bullet PD$ ($R^2 = 0.477$, $p < 0.01$), where 0.009 is a scaling factor relating K_{L_B} to K_L, and PD is the percent of stomata occluded with visible fungal structures. Similar to healthy foliage g_{sref} is directly related to hydraulic sufficiency (K_L), but declines as the presence of *P. gaeumannii* increases in needle stomata.

A second theoretical analysis of equation 1 predicts that the stomatal sensitivity to D (m or slope of the curve, see Figure 3) is equal to:

$$m = -81.131 \bullet K_L + 1.103 \bullet g_{sref} \quad (2)$$

For our field data, we found a significant regression based on the theoretical equation above, where $m = -81.131 \bullet [0.010 \bullet K_{L\_B}] + 1.103 \bullet g_{sref}$

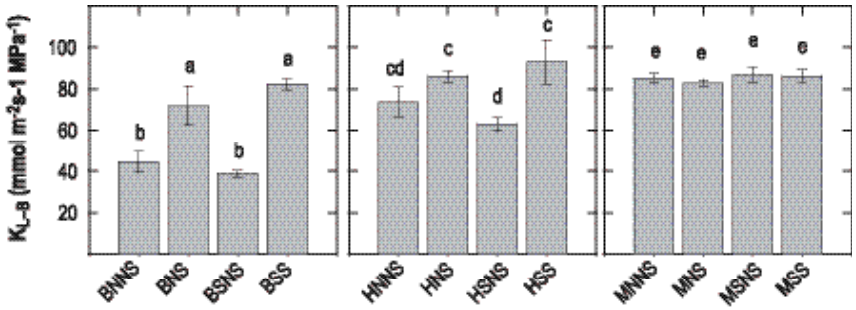


Figure 2. Branch leaf specific conductance at three Douglas-fir plantations (September 2000). The first letter of the site code is the site (B=Beaver, H=Hebo, M=Mac), the second letter is the slope-aspect (N=north, S=south) and the last two are the treatment (NS=nospray, S=spray). Bars are the mean (n=3) and error bars are one standard error. For each site, means with different letters are significantly different (p<0.05) using a t-test

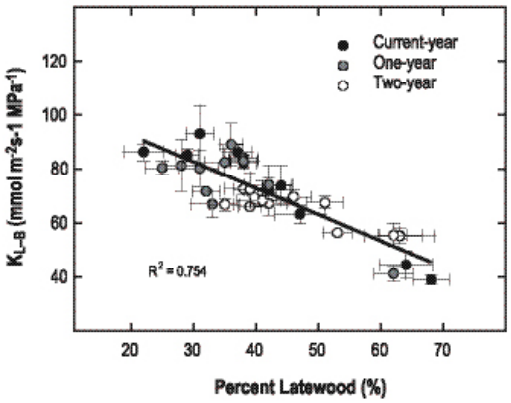


Figure 3. Branch-leaf specific conductance (K_{L_B}) versus the average percent of latewood present in each sample branch from field trees with varying levels of P. gaeumannii infection (September 2000). Each observation is the mean (n=3) and error bars are one standard error.

Table 1. Parameters describing the relationship between g_s and D, disease levels and branch leaf-specific conductance. The function $g_s = b - m\sum \ln D$ was estimated for porometry data from each site-slope-treatment-needle combination by upper boundary line analysis. [1]B, H and M are the Beaver, Hebo, and Mac sites, respectively. [2]N and S are the north and south plots, respectively. [3]NS and S are the nospray and spray treatments, respectively. [4]Age is the year each needle cohort was initiated: 0, 1, and 2 are the current-, 1-, and 2-year-old needles, respectively. [5]b is the y-intercept. [6]m is the slope of the curve. [7]Pseudo is the percent of needle stomata with visible fungal fruiting bodies. [8]K_{L_B} is branch-leaf specific conductance (mmol m^{-2} s^{-1} MPa^{-1}). [9]- missing data due to needle abscission.

						Pseudo[7]		KL[8]	
Site[1]	Slope[2]	Trt[3]	Age[4]	b[5]	m[6]	Mean	SE	Mean	SE
B	N	NS	0	90.6	55.2	0.0	-	44.6	5.2
B	N	NS	1	87.3	25.6	15.0	3.0	55.4	3.0
B	N	NS	2	83.2	25.0	34.4	4.9	55.2	0.8
B	N	S	0	106.2	58.1	0.0	-	72.0	9.2
B	N	S	1	90.3	41.5	0.0	-	74.2	2.5
B	N	S	2	76.6	20.9	27.8	3.3	67.2	0.9
B	S	NS	0	95.5	54.3	0.0	-	38.9	1.7
B	S	NS	1	74.6	31.8	18.6	2.9	41.2	2.9
B	S	NS	2	-	-	-	-	-	4.3
B	S	S	0	106.7	70.0	0.0	-	82.4	2.5
B	S	S	1	90.5	48.1	0.0	-	89.1	7.8
B	S	S	2	-	-	-	-	-	2.4
H	N	NS	0	114.1	69.2	0.0	-	73.8	7.3
H	N	NS	1	111.1	76.0	4.2	1.3	71.7	0.7
H	N	NS	2	101.2	30.9	13.6	3.3	66.0	0.4
H	N	S	0	141.1	89.3	0.0	-	86.2	2.9
H	N	S	1	133.8	81.1	0.0	-	80.4	2.5
H	N	S	2	72.9	34.8	6.3	1.0	66.8	2.3
H	S	NS	0	106.4	65.9	0.0	-	63.2	3.3
H	S	NS	1	105.3	59.7	5.5	2.5	66.9	4.9
H	S	NS	2	125.1	58.8	14.2	2.3	56.4	0.3
H	S	S	0	129.8	84.2	0.0	-	93.0	9.4
H	S	S	1	108.4	60.2	0.0	-	81.3	9.6
H	S	S	2	110.1	48.5	10.0	2.2	72.6	2.3
M	N	NS	0	131.1	92.1	0.0	-	85.2	2.3
M	N	NS	1	131.4	76.2	0.3	0.2	82.5	5.2
M	N	NS	2	75.2	23.1	16.7	5.1	67.5	2.4
M	N	S	0	130.1	88.0	0.0	-	83.0	2.4
M	N	S	1	126.2	86.4	0.0	-	83.0	6.3
M	N	S	2	72.2	15.9	8.8	2.2	72.3	0.9
M	S	NS	0	136.2	81.2	0.0	-	84.0	4.5
M	S	NS	1	129.8	72.1	0.4	0.3	84.0	3.1
M	S	NS	2	72.7	18.8	14.4	2.7	71.0	6.6
M	S	S	0	132.1	78.7	0.0	-	86.3	2.1
M	S	S	1	133.1	88.2	0.0	-	80.2	5.2
M	S	S	2	90.2	44.6	4.4	2.1	68.3	3.0

($R^2 = 0.680$, $p < 0.01$), where 0.010 is an estimated scaling factor relating K_{L_B} and K_L. As was the case for g_{sref}, stomatal sensitivity to D is dependent upon hydraulic limitations and can be modeled according to the balance between water supply and water loss or equation 1.

Summary

Stomatal sensitivity to vapor pressure deficit (D) was investigated in Douglas-fir infected with varying levels of the Swiss needle cast (SNC) fungus, *Phaeocryptopus gaeumannii*. Regardless of infection level, stomatal conductance (g_s) declined exponentially with increasing D. g_{sref} (g_s @ D = 1 kPa) was positively correlated with leaf-specific hydraulic conductance (K_L) and negatively correlated with fungal colonization (number of fruiting bodies present in needle stomata). Despite reduced needle retention in diseased trees, K_L declined due to a reduction in sapwood quantity and quality (*i.e.*, increasing presence of latewood in functional sapwood). In general, stomatal sensitivity to D for all foliage was consistent with stomatal regulation based on a simple hydraulic model ($g_s = K_L(\Psi_{soil} - \Psi_{leaf})/D$), which assumes strict stomatal regulation of leaf water potential. However, when fungal presence reduced maximum g_s below the potential maximum supported by hydraulic architecture stomatal sensitivity was reduced below the theoretical relationship where $dg_s/d\ln D = 0.6 \bullet g_{sref}$.

In previous studies with artificially inoculated seedlings we showed that stomatal occlusion leads to a linear relationship between pseudothecia and maximum stomatal conductance. However, in field trees growth declines and a reduced water supply associated with prolonged disease presence also influence maximum stomatal conductance and regulation. Finally, it is the interaction of these two processes that will influence the total impact of SNC on needle productivity. For example, trees with higher water supply capacities (low LA:SA ratios or latewood) will maintain more open stomata - and carbon productivity - compared to trees with lower water supply capacities and the same level of stomatal occlusion.

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Electrolyte Leakage and Nutrient Status in SNC-infected Douglas-fir

Dan Manter and Jeff Stone

Background

Survival and growth of Douglas-fir is dependent upon adequate sources of assimilated carbon to support metabolic function and deposition of new wood. The available supply of assimilated carbon will be equal to production (i.e., photosynthesis) minus any losses to fungal consumption. Previous work (Manter et al. 2000) has shown that *P. gaeumannii* reduces photosynthesis in infected Douglas-fir trees; however, we do not know the magnitude of fungal carbon consumption. Access to plant metabolites – especially for intercellular pathogens such as *P. gaeumannii* – requires the diffusion of cell contents to the extracellular regions (i.e., outside the cell wall) where the fungus resides. Since this rate of diffusion is dependent upon plant membrane integrity and permeability, some fungi increase the diffusion rates by either physically or chemically (toxin production) altering membranes (e.g., Turner and Graniti 1976, Daub and Briggs 1983, Kwon et al. 1996, Widmer et al. 1998). The end result for the host is not only the loss of cell constituents (e.g., available assimilated carbon), but also a disruption to many of the physiological processes (photosynthesis, respiration, and phloem transport to roots) that rely on membrane-bound proteins (H-ATPases, receptors, carrier and transport proteins) for activity.

Objective

Assess the impact of *P. gaeumannii* infection on host membrane permeability by the electrolyte leakage technique.

Methods

Electrolyte leakage was measured from inoculated Douglas-fir seedlings with varying levels of *P. gaeumannii*-infection. The range of infection levels was achieved by exposing seedlings to natural inoculum for two, four or eight weeks throughout May and June 2000. In December 2000, three branches from each of six seedlings per exposure group were harvested. One-year-old needles from each seedling were pooled and electrolyte leakage was measured by conductivity on a sub sample of 50 needles. Fungal colonization was determined by counts of pseudothecia density and, and quantitative PCR for a sub sample of 10 needles.

Electrical conductivity measurements were obtained by placing 50 needles in a test tube with 10 ml of 0.01 % tween 20 (0.1 ml tween 20 in 1 L H_2O). After 24 hours, electrical conductivity (EC) was measured for each test-tube. Needles were then autoclaved for 30 minutes. After 24 hours total electrical conductivity (TEC) was re-measured. Relative conductivity (RC) was then calculated [EC/TEC].

Results & Discussion

In other studies, relative conductivity has been shown to be a successful indicator of membrane damage and permeability (Calkins and Swanson 1990). In the case of SNC, relative conductivity measurements show no indication of a significant impact of fungal infection on membrane permeability (Figure 1). The slight increase in relative conductivity is well-below values observed in other fungal diseases that cause significant damage to host membranes (25% or more) (e.g. Kwon et al. 1996, Widmer et al. 1998).

One inherent problem with electrical conductivity assay is that it is not specific; it detects leakage from both fungal and host tissues. Therefore,

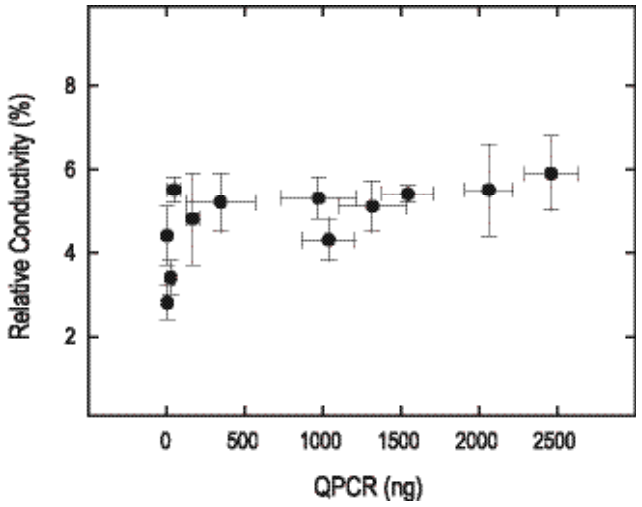

Figure 1. Relative conductivity as P. gaeumannii *infection increases. QPCR is quantitative PCR.*

based on determinations from five *P. gaeumannii* isolates, we estimated the amount of electolyte leakage contributed by the fungus and divided the electrolyte leakage components of infected needles into host and fungal components. This was achieved by determining the relationship between fungal biomass, quantitative PCR and electrolye leakage (Figure 2), so that an estimate of the fungal electrolyte leakage (EC_F) could be determined from the needle PCR results [EC_F = 35.24*PCR/1243.1]. Finally, the needle only electrolyte leakage (EC_N) was determined [EC_N = EC – EC_F].

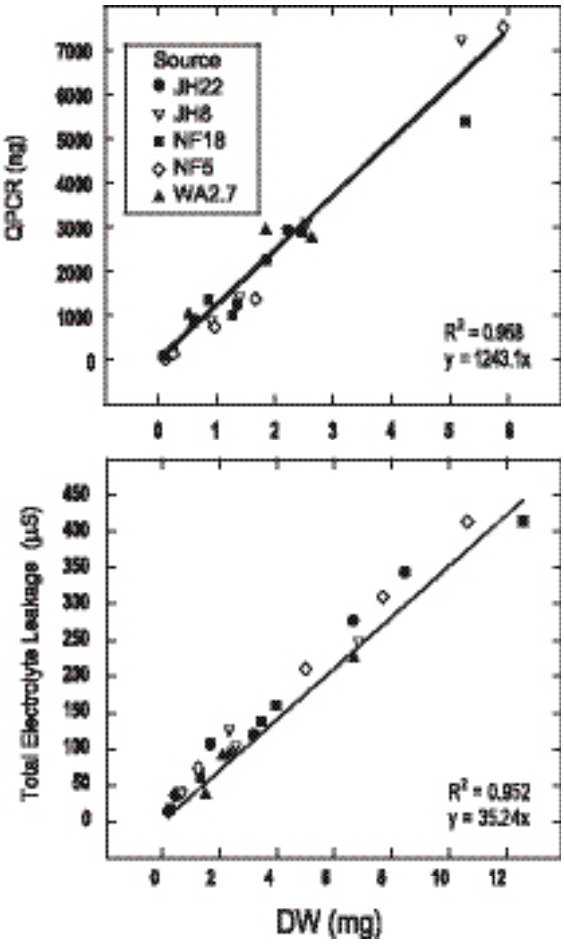

Figure 2. Relationship between quantitative PCR, electrolyte leakage and P. gaeumannii *biomass (DW) for five single spore isolates.*

Figure 3 shows the contribution of the fungus and host to the electrolyte leakage levels. As *P. gaeumannii* infection increases electrolyte leakage increases, most likely due to the increasing fungal biomass and leakage from its own membranes. Whereas, for the host, the estimated leakage declines, suggesting that the host has fewer electrolytes available for leakage. In other words, the fungus appears to be consuming a significant portion of the host electrolytes.

In conclusion, *P. gaeumannii* does not have a significant impact on host membrane integrity and permeability. Based on this result and our failed attempts to recover any fungal toxins (Manter et al. 2000), the first and major impact of *P. gaeumannii* on host physiology is the production of pseudothecia and occlusion of stomata, not the disruption of physiological processes by membrane damage.

Since *P. gaeumannii* must obtain its carbon and energy source from its host, our results suggest that this occurs by

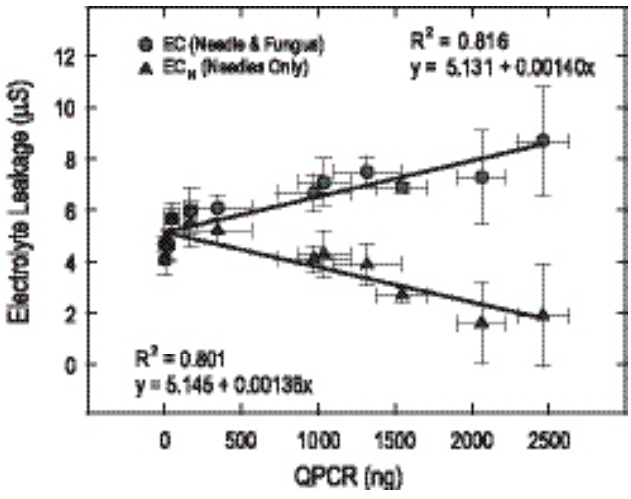

Figure 3. Electrolyte leakage from P. gaeumannii *-infected Douglas-fir foliage. EC is the observed leakage and represents needle and fungal leakage. EC_N is the estimated leakage from the needle membranes only. QPCR is quantitative PCR.*

passive interception of host photosynthate in intercellular spaces. Diffusion of carbon substrates across the host cell membrane is not increased due to the activity of fungal metabolites that alter membrane permeability. The apparent decrease in electrolytes from the needle component with increasing fungal biomass suggests that one effect of fungal colonization is to reduce the pool of available fixed carbon that might otherwise be translocated from foliage to build wood or root tissue.

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Post-Inoculation Environment Studies

Dan Manter, Paul Reeser, and Jeff Stone

Background

Several climatic factors have been suggested to influence the development of Swiss needle cast disease. In considering the contrasting behavior of *P. gaeumannii* on its host in western North America, where it was considered a harmless parasite, versus Europe, where it was a serious defoliating pathogen, Boyce (1940) concluded that seasonal differences in local climate were the most probable factors affecting fungal growth and development. In previous studies we have observed that fungal colonization increases faster in upper canopies and southerly exposed foliage (Manter 2000). Since several climatic variables co-vary with these positional effects (temperature, light, relative humidity, and plant water content), we implemented a study to investigate several of these factors individually.

Objectives

To understand the post-inoculation environmental factors influencing *P. gaeumannii* fungal growth and colonization in field trees.

Methods

Douglas-fir seedlings (Burnt Woods open-pollinated seed source, Starker Forests, Inc.) were exposed to natural inoculum at a heavily diseased coastal Oregon plantation for 4 weeks in June 2000. After inoculation seedlings were incubated under different conditions of shade, irrigation, and mist, and fungal colonization was determined at monthly intervals by quantitative PCR and pseudothecia counts. Post-inoculation environment treatments were applied in a split-randomized complete block design with overhead mist and shade treatments applied to the whole plot, and irrigation treatments applied to the sub-plot (Figure 1). Mist treatments were no mist or three 2 hr mist treatments daily, shade treatments were 100 or 50 % of ambient light, and irrigation treatments were 0.5 or 0.125 gallons of water per day. Incubation chambers were frames constructed from PVC pipe to support shade cloth and mist tubing. Dimensions of the chambers were 3 x 6 x 4 ft. Each chamber held 6 trees, and the two irrigation treatments were applied to 3 trees in each chamber. There were 3 blocks of 4 chambers/block.

Results & Discussion

Based on the final levels of *P. gaeumannii* colonization, the sun and

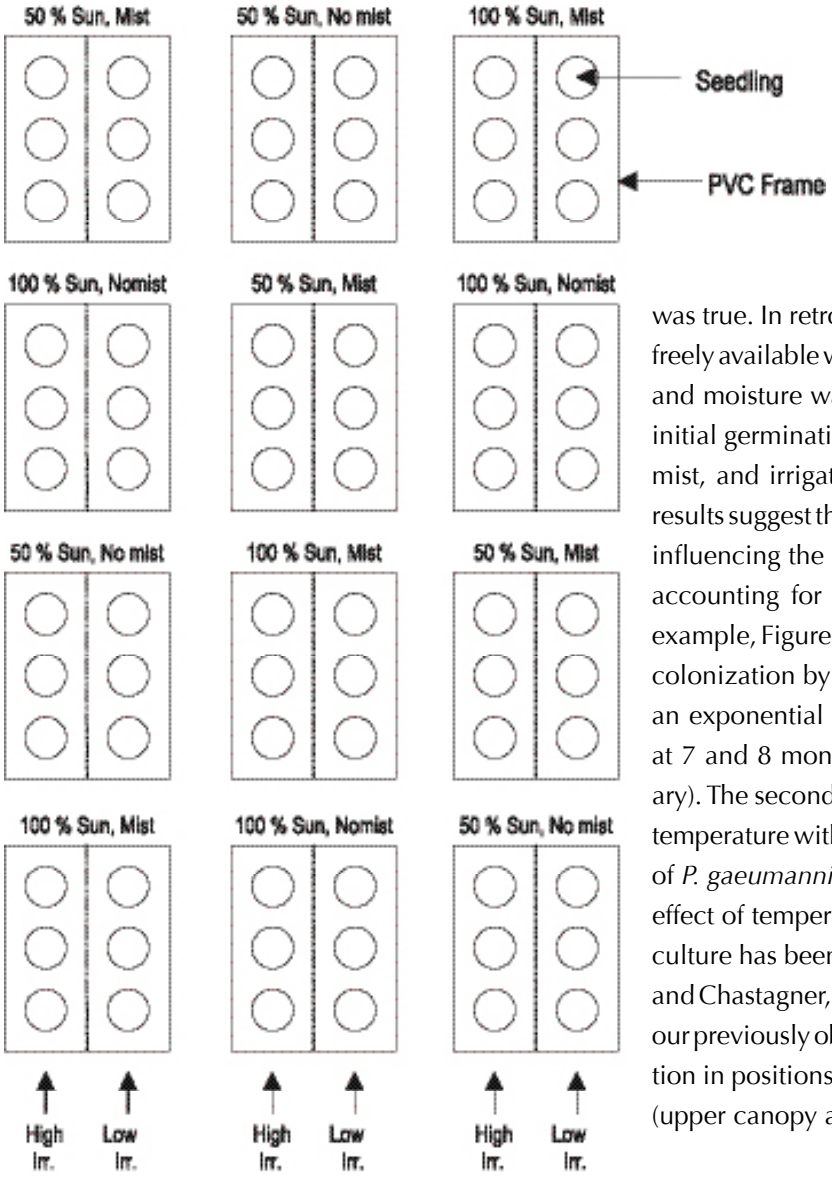

Figure 1. Split-randomized-complete block design.

mist treatments had a significant effect on *P. gaeumannii* growth (Figure 2). Colonization was greatest under the high light and low mist treatments, and was lowest under the shade/high mist. Despite a significant impact on plant water stress (data not shown), the irrigation treatment did not influence *P. gaeumannii* growth (Figure 2). Since all trees in the study received the same exposure to inoculum, the different post inoculation incubation conditions must have affected the rate of foliage colonization by *P. gaeumannii*.

Considering the climate conditions of the coastal Oregon area where SNC is most severe, we had expected fungal colonization to be greater under the mist treatment; however, the opposite was true. In retrospect, this is not surprising since water is freely available within needles where the fungus is growing, and moisture was not limiting at the field site during the initial germination and infection period. Although shade, mist, and irrigation were the primary factors tested, our results suggest that temperature may be the principle factor influencing the rate of fungal colonization of foliage and accounting for the differences between treatments. For example, Figure 3 shows the monthly progression of fungal colonization by treatment. For all, colonization followed an exponential growth curve with the greatest increases at 7 and 8 months after inoculation (January and February). The second panel of Figure 3 shows that as the mean temperature within each treatment increases the final level of *P. gaeumannii* pseudothecia production increases. The effect of temperature on growth of *P. gaeumannii* in pure culture has been documented (Capitano, 1999; Michaels and Chastagner, 1984). Finally, this result is consistent with our previously observed patterns of greater fungal colonization in positions where ambient temperatures are warmer (upper canopy and southerly exposed foliage).

Future Studies

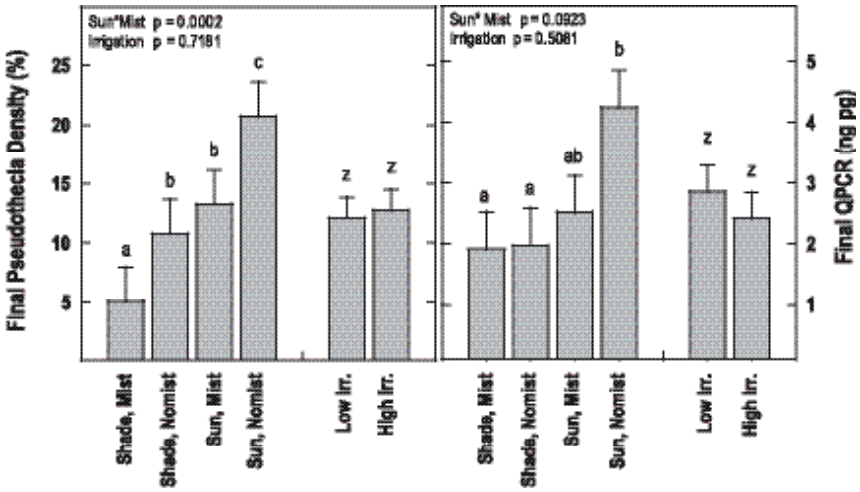

Figure 2. Final pseudothecia density and QPCR by treatment.

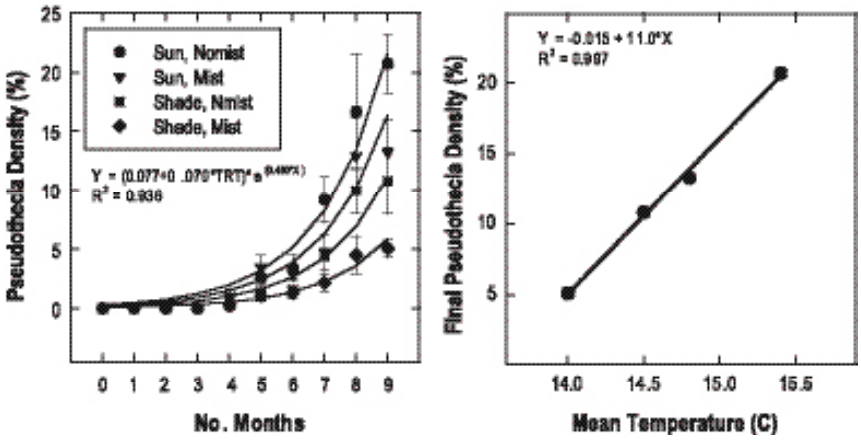

Figure 3. P. gaeumannii fungal colonization over time under the various incubation treatments (0=June 2000 and 9=March 2001), and the relationship between final pseudothecia density and treatment temperature.

To validate the apparent temperature effect we are currently conducting a second post-inoculation environment experiment. Like the current experiment a monthly assessment of fungal colonization will be conducted; however, post-inoculation treatments will be limited to three different temperature regimes in the greenhouse.

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Early Testing Of Douglas-Fir For Swiss Needle Cast Tolerance

Fatih Temel and Randy Johnson

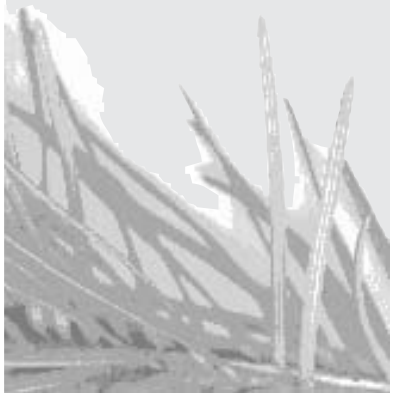
Abstract

Foliage health traits, growth and measures of SNC infection were examined in families of Douglas-fir at juvenile (age-2) and mature (age 10 and 12) ages. There was significant genetic variation in most traits representing SNC symptoms; therefore, breeding for improved foliage health is possible. Early selection at age 2 was 25 to 100% as efficient as waiting until age 10 or 12. Because there was no difference among families for SNC infection (% SNC fungus in needles or % of occluded stomata) it appears that we are breeding for tolerance to SNC, not resistance per se. The better families had a tendency to shed heavily infected needles before the poorer families.

The broad objectives of this study were to obtain an understanding of the genetics of disease resistance to Swiss needle cast (SNC) and then to develop an early screening procedure for detecting resistance in seedlings or young trees. Specific objectives were to:

- investigate genetics of foliage traits that may be indicative of resistance to SNC,
- develop a method to inoculate seedlings which would produce variation in disease symptoms,
- determine reliability of tolerance prediction in older trees from seedling data, and
- determine the efficiency of early selection for SNC traits in Douglas-fir.

Fifty-five wind-pollinated Douglas-fir families were included in this study. We established two seedling progeny test plantations on Simpson Timber Company land using 1-0 seedlings in Pleasant Valley near Tillamook and in Toledo near Newport in 1999. The plantations were surrounded with heavily infected Douglas-fir stands. We relied on *Phaeocryptopus gaeumannii* spores released from these stands for inoculation of our trees in the plantations. We also tried artificially inoculating one set of seedlings at Dorena Tree Improvement Center by suspending infected Douglas-fir branches over our seedlings in the spring of 2000. Although we observed infections, we did not see any symptom development in the following summer. Thus, we only report on data collected from the two field progeny plantations.



Trees at these plantations (i.e., juvenile) were visually scored for SNC traits (Table 1) in the summer of year 2000. In addition, needle samples

were collected for further laboratory assessments in an attempt to find other traits that might better represent disease tolerance in Douglas-fir. Two older (i.e., mature) progeny test plantations (Salal and Gordy) were also visually assessed in the field for SNC traits in springs of 1996 and 1998, when the trees were 10 and 12 years old.

On average, the disease symptoms were more severe and had larger family differences in Pleasant Valley than in Toledo. However, overall means for proportion of stomata occluded with pseudothecia and fungal DNA were higher in Toledo. The mature test sites had similar symptom severity, but variation was higher on the Salal site.

Genetics of the traits and interrelationships among them were investigated by estimating individual narrow-sense and family-mean heritabilities, and genetic and phenotypic correlations among traits. Heritability estimates the degree of genetic control over the expression of trait. For example, an individual narrow-sense heritability estimate of 0.25 implies that 25% of the variation we see among individuals is controlled by genes which are passed from parent to offspring. A family-mean heritability of 0.25 would imply that 25% of the variation in family means is controlled by genetics, the remainder being environmental variation or that associated with genetic effects not passed on from parent to offspring. Genetic correlations indicate the degree to which the genes for one trait affect another. It also represents how breeding for one trait will affect another.

For both juvenile and mature plantations, data were combined over the two progeny plantations in each age group (juvenile and mature). In addition, for mature sites, the age-10 and age-12 assessments were averaged. Thus we could easily use all the available data in a single analysis.

For all traits under investigation, except for the amount of fungal DNA, families differed significantly (p=0.05). The variation in these traits was under low to moderate genetic control as evidenced by the low to moderate estimates of individual heritability (Table 2). Individual heritability estimates for field traits ranged from 0.11 to 0.37 and ranged from 0.33 to 0.73 for family means. Similar patterns of variation were evident in both the juvenile and mature trials for all traits except needle retention. The family mean heritability estimate for this trait at the mature age was considerably lower than that of the juvenile age because the families did not perform the same at the two mature sites (i.e., a relatively poor family mean correlation between the two sites).

For the two SNC traits assessed both in the field and in the laboratory at the juvenile age (needle color and needle retention), the heritability estimates for needle retention were

Table 1. Descriptions of traits and measurement methods.

Trait	Description and measurement method
Juvenile (field)	Assessed in Summer 00′.
Needle color	Visually scored on a south facing single internode on a scale from 1 (yellow) to 3 (green)
Foliage color	Visually scored over entire seedling on a scale from 1 (yellow) to 3 (green).
Needle retention	Visually scored on the same internode as above on a scale from 0 (0-10% retention) to 9 (91-100% retention).
Foliage density	Visually scored over entire seedling on a scale from 1 (sparse) to 6 (dense).
Juvenile (lab.)	Assessed on a sample internode collected from the south side of each seedling in Summer 00′.
Needle color	Visually scored on the sample internode by comparing it to a color photograph of 4 different needle colors (1=yellow to 4=green).
Retention	Visually assessed from 0 (from 0 (0-10% retention) to 9 (91-100% retention).
Dry weight (g)	Dry weight of selected 50 needles.
Needle length (mm)	Length of 10 randomly selected needles (included in the 50 above).
Fungal DNA (pg P. gaeumannii DNA/ ng Douglas-fir DNA)	Amount of fungal DNA in sampled needles.
Pseudothecia (%)	Percent stomata clogged with pseudothecia, assessed on the central 50 stomata of the above 10 needles in the second stomata row at the right side of needle midrib needle petiole facing observer.
Specific area (cm^2/g)	Projected needle specific area calculated as Fresh weight/projected needle area.
Mature	Assessed in 1996 and 1998.
Crown density	Visually assessed from 1 (sparse) to 6 (dense).
Foliage color	Visually assessed from 1 (yellow) to 3 (green).
Needle retention	Visually assessed from 0 (0-10% retention) to 9 (91-100% retention).

Table 2. Individual (I) and family mean (F) heritability estimates for Swiss needle cast traits in juvenile and mature sites.

	Juvenile		Mature	
Field traits	I	F	I	F
Needle color	0.11	0.49*	NA	NA
Foliage color	0.14	0.50	0.21	0.52
Needle retention	0.20	0.60	0.13	0.33*
Foliage density	0.20	0.64	0.37	0.73
Laboratory traits				
Needle color	0.06	0.26		
Needle retention	0.21	0.57		
Dry weight	NA	0.40		
Needle length	0.16	0.45		
Fungal DNA	NA	NS		
Pseudothecia	0.14	0.43		
Needle specific area	NA	0.41		

* Significant family-by-site interaction (p=0.05).

NA: Not available.

NS: No significant family differences.

similar for the two assessments, but the heritability estimates for needle color were lower in the laboratory assessment.

Genetic correlations between laboratory and field assessments of needle color and needle retention were near unity, implying that they are both the same trait controlled by the same genes. This indicates that either kind of assessment could be employed. Genetic correlations of the lab and field juvenile traits with the mature traits were the same for needle retention but stronger and more significant for needle color when the juvenile trait was assessed in the field. Since field assessments are much simpler and easier to conduct than laboratory assessments, our choice is to assess these traits in the field.

Among the other traits we assessed in laboratory only percent stomata occluded with pseudothecia and needle specific area had significant genetic correlations with SNC traits assessed in the juvenile field trials. Percent stomata occluded with pseudothecia significantly correlated with needle retention (0.66) and foliage density (0.62). A low negative genetic correlation was observed between needle specific area and needle color assessed in the field (-0.37). No significant genetic correlations between these five laboratory traits and SNC traits assessed in the mature sites were observed, except for between dry weight and foliage color (-0.72) and needle length and foliage color (-0.72). These two traits, however, are not likely to be good candidates to be employed in early testing efforts due to labor required assessing them and the existence of other juvenile field SNC traits (needle color and foliage color) that are also genetically correlated with mature SNC traits (Table 3). Therefore, we will no longer discuss any of the traits that we assessed in the laboratory in this report.

There were significant positive genetic correlations among SNC traits at each age. Correlations were stronger for juvenile traits (mean=0.75) than for mature traits (mean=0.56). Genetic correlations between comparable juvenile and mature traits were highest for needle retention, moderate for color and low (and non-significant) for foliage density (Table 3).

In order to determine efficiency of early selection for Swiss needle cast tolerance in Douglas-fir, expected gains from selection of SNC traits at the mature age and correlated responses from selection of correlated traits at the earlier age were estimated. The relative efficiency of early selection was calculated as the ratio between gains estimated in the mature traits from selections made at juvenile and mature ages.

Early selection appears to be as efficient as later selection for needle retention and fifty percent as efficient for foliage color (Table 4). These gains from early selection come eight years earlier than waiting until age 10.

Our results from this study indicate that natural inoculation and visual assessment of the disease symptoms were adequate for early testing purposes. Low to moderate genetic control over the severity of disease symptoms and presence of significant genetic correlations between juvenile and mature plantations make early

Table 3. Genetic correlations among juvenile (in italic) and mature (in regular) SNC traits and between the ages (in bold) for the same traits.

	Needle color	Needle retention	Foliage density	Foliage color
Needle retention	0.61	**0.75**	0.49	0.76
Foliage density	0.56	0.95	**0.30**	0.42
Foliage color	0.88	0.81	0.70	**0.50,0.53**

All correlations are statistically significant at p=0.05 lever, except for foliage density (0.30) and foliage color (0.50) between the ages.

Table 4. Relative efficiencies of early selection in SNC traits.

Juvenile trait selected	Response in mature traits: Foliage color	Needle retention	Foliage density
Needle color	**0.52**	0.91	0.16
Foliage color	0.49	1.02	0.24
Needle retention	0.45	**1.01**	0.23
Foliage density	0.63	0.87	0.28

selection possible for foliage color and needle retention.

Relationship Between Level of Phaeocryptopus gaeumannii Infection and SNC Symptom Severity

In a second study we investigated six wind-pollinated Douglas-fir families at two 15-year old progeny test plantations (Acey Creek and Coal Creek) to explore the relationship between SNC symptom severity and level of *P. gaeumannii* infection.

Two families were selected from each disease severity group (i.e., low, moderate and severe) based on previous SNC and growth assessments conducted on these sites. A total of 108 trees from these families were assessed for DBH, branch size, foliage color and crown density in the field (Table 5). A branch located at the south side of the 4th whorl from the top was cut and its diameter was measured. Later, needle color and retention were scored for the last four growing seasons separately on this branch (Table 5). Three internodes for each of the last two growing seasons (1998 and 1999) were then clipped from this branch and transported to the laboratory. Needles on these internodes then were assessed for the traits listed in Table 5. Only two lateral internodes per growing season were assessed because the leaders had unusual needle retention.

We used analysis of variance (ANOVA) to detect family differences in the traits assessed. The ANOVA model examined the effects of family type (i.e., low, moderate and severe), site and replication. Regression analysis was employed to see how actual level of infection affected the level of symptom expression. Field SNC traits were considered as SNC symptoms. The amount of *P. gaeumannii* in 1998 and 1999 foliage (or their average) and the percent stomata occluded with pseudothecia were considered as levels of infection.

There were significant family differences for all traits assessed in the field, except for foliage color. Among the traits assessed in the laboratory, families differed only in fresh weight of sampled needles. There were significant differences between 1998 and 1999 foliage for all traits assessed in the laboratory, except for needle length. As in the earlier study, we did not see any significant differences

Table 5. Traits assessed and assessment methods for the infection level and symptom severity study.

Traits	Assessment method (abbreviation)
Field	
DBH (mm)	Diameter of the tree measured at the breast height.
Branch diameter (mm)	Diameter of the sampled branch measured at the base.
Needle retention	Visually scored from 0 (0-10% retention) to 9 (91-100% retention) for the last 4 growing seasons on the sampled branch.
Needle color	Visually scored from 1 (yellow) to 3 (dark green) for the last 4 growing seasons on the sampled branch.
Foliage color	Visual color score of overall tree crown from 1 (yellow) to 3 (dark green).
Crown density	Visual score of foliage density from 1 (sparse) to 6 (dense).
Branch size	Visual score of branch size of a tree from 1 (smallest) to 4 (largest).
Laboratory	These traits were assessed for foliage of 1999 and 1998 separately.
Needle color	Average visual color score of the two sample internodes scored from 1(yellow) to 4 (dark green) by comparing the samples with a color photo of 4 sample foliages.
Percent stomata occluded with pseudothecia (%)	Percent stomata occluded with pseudothecia on the 10 needles.
Needle length (mm)	Average length of the 10 needles.
Internode length (cm)	Average length of the two sample internodes.
Needle retention (%)	Average needle retention on the two sample internodes obtained by dividing total number of needles to total number of needle scars.
Projected needle specific area (cm^2/g)	Obtained by dividing projected area to fresh needle weight.
Fresh weight (g)	Fresh weight of the 50 needles.
Dry weight (g)	Air-dried weight of the 50 needles.
Moisture content (g)	Difference between fresh weight and dry weight.
Fungal DNA (pg P. gaeumannii DNA/ ng Douglas-fir DNA)	Amount of fungal DNA obtained by quantitative PCR.

among families for fungal DNA content, even though the most extreme families with regard to symptom severity were investigated.

Among all SNC symptoms, only needle retention was related to fungal presence and family. The relationship was:

Foliage Needle Retention =2.88 + 0.0032(*DNA99*) + 1.421(*FAMTYPE*) – 0.224(*DNA99*FAMTYPE*).

Although this model only accounted for 15% of the total variation, coefficients of all explanatory variables were significant (p=0.05), except for DNA.

Graphical explanation of this model is shown in figure 1. Although there was no significant difference among families with respect to *P. gaeumannii* DNA content in 1999 foliage, there is an obvious pattern in each family type when needle retention is plotted against this variable. In families where the disease symptoms were light, trees with lower needle retention scores had increased amounts of *P. gaeumannii* DNA in their needles. Conversely, in the severely affected families, trees with higher needle retention scores had increased amounts of *P. gaeumannii* DNA. In the middle group no obvious pattern was detected. These observations imply that better performing families cast their infected needles, while severely effected families try to hold on to them longer. The heavily infected needles that the poorer families are retaining are a drain on the tree; the better families appear to be shedding these needles when they are no longer contributing.

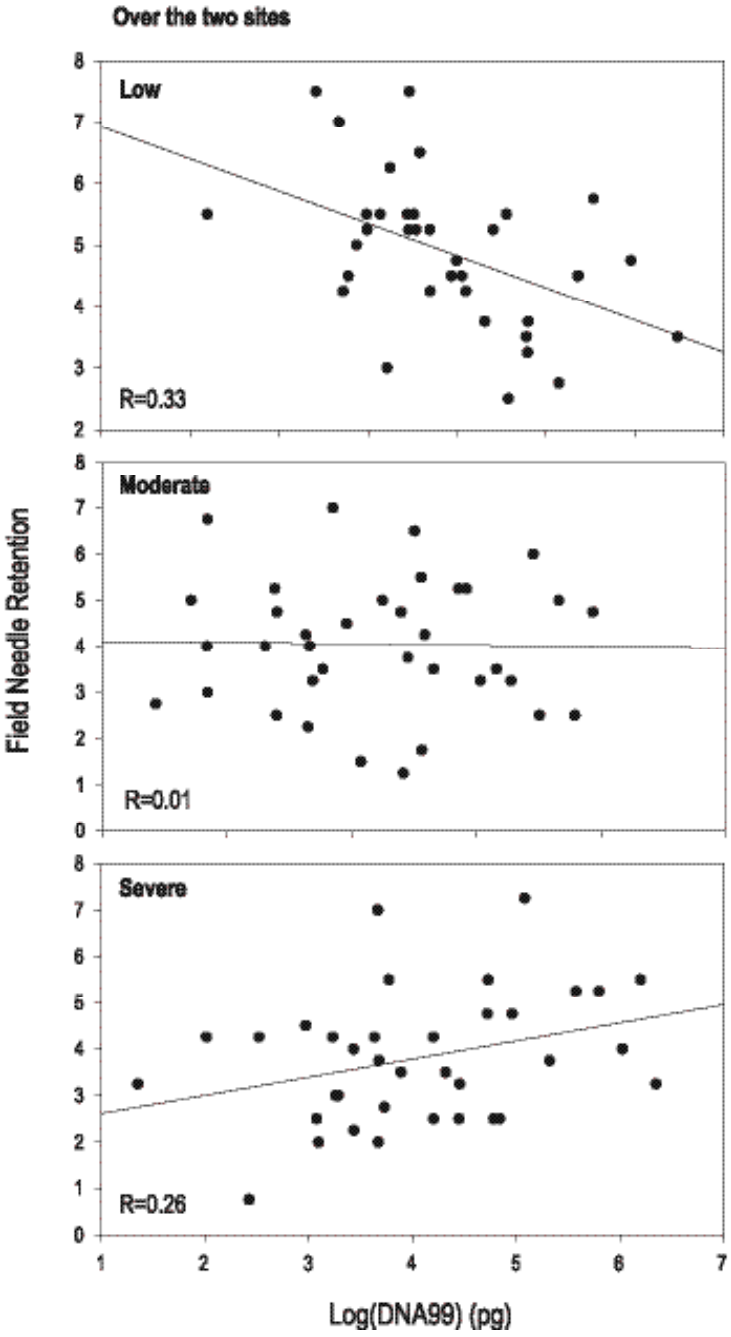


Figure 1. *Relationship between log(fungal DNA content in 1999 needles) and field needle retention.*

Tolerance and resistance are two mechanisms of plant defense against diseases. In the case of resistance, host and the pathogen are more or less incompatible with one another. Host organism either physically blocks invasion by pathogen or chemically reacts to it after infection. In tolerance, on the other hand, plants are able to produce good crop even when they are infected by the pathogen. Results of the regression analysis and examination of figure 1 indicate that the fungus seems to be infecting and colonizing every tree at a similar pace. Some families, however, are able to minimize symptom expression and grow unaffected by the disease. All these evidence suggest that Douglas-fir's mechanism of dealing with SNC is probably tolerance, not resistance.

Effect of seed source on infection and colonization of Douglas-fir foliage by Phaeocryptopus gaeumannii

Jeffrey Stone and Paul Reeser

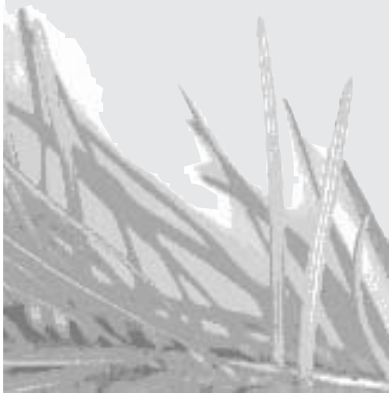
ABSTRACT

Variation in susceptibility to infection by *Phaeocryptopus gaeumannii* was observed in eleven Douglas-fir seed provenances from western Oregon and northern California. No relationship between seed zone or elevation and susceptibility was found. Differential susceptibility was seen between two groups of seedlings from the same seed source that had been grown under different nursery conditions.

INTRODUCTION

Variation in susceptibility to infection by *Phaeocryptopus gaeumannii* and Swiss needle cast disease among Douglas-fir provenances has been documented for relatively large scale geographic distributions (Hood 1982, McDermott and Robinson 1989, Stephan 1997). Hood (1982) surveyed the incidence of *P. gaeumannii* in southern British Columbia and found much higher levels of infection in the coastal regions than in the dry interior. In the same paper however, Hood (1982) sampled trees in a provinance selection study at Cowichan Lake, B.C. and found that provenances from the coastal areas of British Columbia, Washington and Oregon had significantly lower incidences of infection than provenances from east of the Coast Range and northern California. Although incidence of infection was less in trees growing in the dry interior, provenances from those areas were more susceptible to infection compared to coastal provenances.

McDermott and Robinson (1989) investigated incidence of *P. gaeumannii* and SNC in another provenance plantation near Vancouver, and found a similar relationship of higher incidence of infection in provenances from northern California and interior B.C. than from coastal Oregon, Washington and British Columbia. The general pattern of greater susceptibility to SNC in Douglas-fir originating from drier locations, also observed by Hood (1982), led McDermott and Robinson (1989) to hypothesize that there is an endemic level of resistance to SNC in natural populations of Douglas-fir that is related to the regional level of disease pressure. More favorable conditions for the growth of the fungus and high disease pressure, generally related to areas of higher rainfall, has led to selection for increased disease resistance in genotypes from these areas. This hypothesis was generally supported by the findings of Stephan (1997) who compared



abundance of pseudothecia on Douglas-firs in a provenance plantation in northern Germany, where *P. gaeumannii* has been introduced (Boyce 1940). Douglas-fir from thirty-one provenances representing the natural range of Douglas-fir in North America were included in the study. Stephan (1997) found the highest numbers of pseudothecia on one- and two-year-old needles on the interior form (grey variety) of Douglas-fir from provenances from interior British Columbia. Representatives of coastal form (green variety) Douglas-fir generally had less abundant fruit bodies than interior form trees, but there was a high degree of variation in infection among the coastal form trees.

The findings of McDermott and Robinson (1987) and Stephan (1997) suggest a high degree of genetic variation in Douglas-fir with respect to susceptibility to infection by P. *gaeumannii*, although the physiological basis for susceptibility or resistance is not known.

Both of these studies were concerned with variation in susceptibility to infection in Douglas-fir over a broad geographic area. The study by Stephan (1997) suggests that there may also be variation in susceptibility within the coastal form of Douglas-fir, but to date there have been few investigations of variation in disease susceptibility in provenances from Coastal Oregon and Washington (Kastner et al. 2001). This study was undertaken to compare susceptibility to infection of Douglas-fir from coastal Oregon, Washington, and northern California provenances. A secondary objective was to determine whether seed zone and elevation of origin are related to susceptibility to infection in these provenances.

MATERIALS AND METHODS

Seedlings from eleven different seed zones and elevations were exposed to natural inoculum at the Salal study site between March 14 – July 7, 2000, before bud break. Twenty seedlings of each seed source were grouped in four blocks with five replicate seedlings per block. Seedlings were then returned to the OSU Botany Field Lab for incubation and kept in the same blocks. Seedlings were obtained from Pelton nursery, Phipps nursery, and the J.H. Stone nursery. Seed zones and elevations represented in the study are listed in Table 1.

Incidence of *P. gaeumannii* and pseudothecial abundance were determined from foliage collected in April, 2001. Two shoots bearing needles of the 2000 needle complement were clipped, the needles stripped from the shoots, and a sample of 50 drawn randomly. Needles were affixed to 3 x 5″ index cards with double-sided adhesive tape for examination. Infection incidence was determined as the proportion of needles bearing pseudothcia of *P. gaeumannii*. Pseudothecial density was determined from a subset of 10 needles bearing pseudothecia. The number of pseudothecia in a band of stomata from the petiole, middle, and apical portions of the needle was counted under a dissecting microscope fitted with a counting grid. The total pseudothecia in the three segments were averaged for each needle and the average of ten needles calculated for each tree. Infection index is a measure of the overall infection severity and is the product of incidence, i.e. the proportion of infected needle, and pseudothecial density.

RESULTS

Differences in infection incidence and pseudothecial density were found among the eleven seed

Table 1. Sources of Douglas-fir seedlings used in this study.

Seed Zone	ID	Elevation	Source	Locale
2	NNC0124DFR98	1000	Phipps	South Coast Range
1	NNC0125DFR98	2000	Phipps	South Coast
5	NNC2524DFR98	1500	Phipps	North Coast
5	NNC0524DFR98	500	Phipps	North Coast
4	NNC1425DFR98	1500	Phipps	Central Coast
4	NNC0424DFR98	1000	Phipps	Central Coast
4	32021		Pelton	Burnt Woods
4	32021 "special"		Pelton	Burnt Woods
	N. California		Simpson	N. California
1	11-11062-1525-93-SIA	1500-2500	J.H. Stone	Coast Siskyou
15/501	NNC5525DFR98	4000	Phipps	Rogue/Cascades

sources tested (Figs. 1, 2, 3). Incidence of infection ranged from 0.63 – 0.96, indicating that all seed sources were susceptible to infection (Fig 1.). However, there were significant differences in infection incidence, with the highest incidence occurring in the seed zone 4 "Burnt Woods", seed zone 4/ 1500' and seed zone 5/ 500' seed sources. The lowest incidence occurred the seed zone 1/ 2000'.

Pseudothecial density also varied among the seed sources (Fig. 2). Pseudothecia were most abundant on foliage of the seed zone 4, which had a mean of 30% of stomata occluded on one-year –old needles. Seedlings from the seed zone 1/ 2000' seed source had the lowest pseudothecial density of about 10%. Infection index, the product of incidence x pseudothecial density gives a measure for ranking seed sources by overall susceptibility to infection (Fig. 3). The infection index identifies a highly susceptible group, the 32021 "special", an intermediate group comprised of a northern California source, 32021 standard, seed zone 5 /500', and a low susceptibility group comprised of two sources from zone 2/ 2000', and sources from zone 2 /1000' and zone 5 /1500'.

DISCUSSION

All trees were exposed to the same inoculum source concurrently and arranged in a randomized block during both inoculum exposure and subsequent incubation. These precautions should have minimized effects of variation in inoculum exposure and microhabitat conditions experienced by individual trees. The results there-

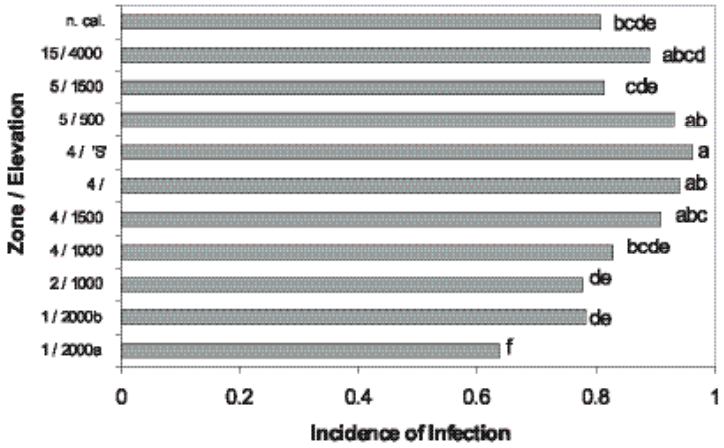


Figure 1. Proportion of needles bearing pseudothecia of Phaeocryptopus gaeumannii *in eleven seed sources tested. Means followed by the same letter are not different at P > 0.05 (Fisher's LSD).*

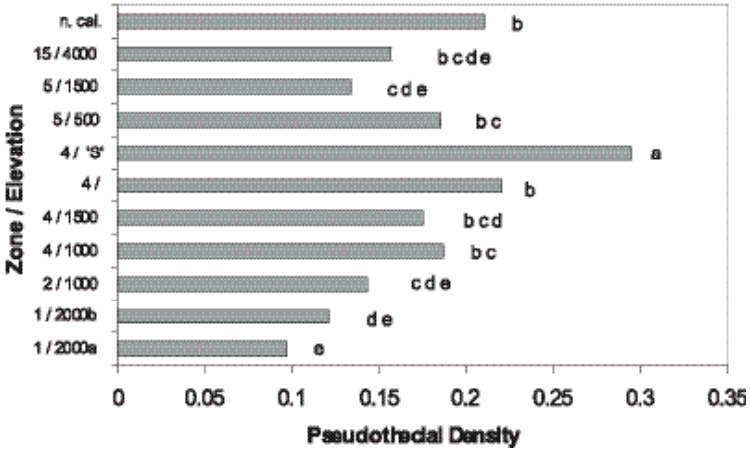


Figure 2. Proportion of stomata occluded by pseudothecia of Phaeocryptopus gaeumannii *in eleven seed sources tested. Means followed by the same letter are not different at P > 0.05 (Fisher's LSD).*

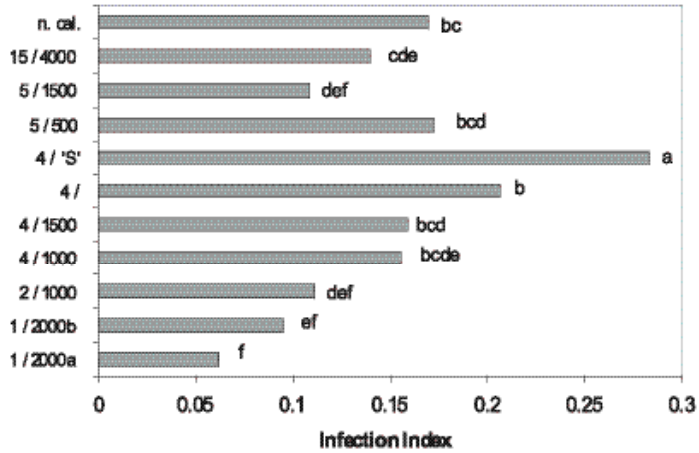


Figure 3. Infection index (incidence x pseudothecial density) of Phaeocryptopus gaeumannii *in eleven seed sources tested. Means followed by the same letter are not different at P > 0.05 (Fisher's LSD).*

fore support the conclusion that significant variation exists within coastal Douglas-fir seed sources. There was no apparent relationship between zone or elevation and susceptibility to disease, which appears to contradict the prediction of McDermott and Robinson (1979). Contrary to the hypothesis proposed by McDermott and Robinson (1979), susceptibility to infection was lowest in the relatively drier, more southerly zone 1/2000' and zone 2/1000' sources, which have comparatively lower disease pressure than the lower elevation sources from zones 4 and 5.

However, the variation in susceptibility to infection observed here may not reflect only genetic variation. It was an unexpected result that the group with the highest susceptibility to infection, the Burnt Woods 32021 "special" differed significantly from 32021 standard. These two groups differed only in the conditions they were cultivated under in the nursery. Both groups had statistically equivalent incidence of infection of around 95%, but a significantly greater number of pseudothecia were produced on the 32021 "special" group. The higher pseudothecial density seen on the 32021 special group probably reflects a higher proportion of successful ascospore infection of this foliage. Previous studies on inoculum exposure have shown that increased exposure to inoculum leads to higher levels of pseudothecium production even when incidence approaches 100% (Stone et al. 2000). The differences in susceptibility to infection in 32021 "special" and 32021 standard suggest that cultural conditions as well as genotype can influence susceptibility to Swiss needle cast. It is not surprising that culture conditions could affect disease susceptibility, however, the magnitude of the difference in relation to genetic differences is surprising. Since seed sources used in this study were obtained from different nurseries, differences in cultivation conditions may account for some of the differences observed among the seed sources. Effects due to genetic variation and effects due to cultivation conditions cannot be separated.

The basis for differences in susceptibility to infection by *P. gaeumannii*, whether due to genetic differences or cultural conditions, is not known. Greater variation was observed in pseudothecium production than infection incidence, suggesting that either the proportion of ascospores that sucessfully infected the needles or rate of fungal growth after infection differed in some of the seed sources. Significant between-tree variation in infection levels also has been observed for old-growth Douglas-fir at the Wind River canopy crane research site (Stone and Kerrigan unpubl). Although the infection biology of *P. gaeumannii* on Douglas-fir has been documented (Capitano 1999), factors that might differentially affect the rate of successful infection by ascospores are insufficiently understood. It is known that needles are only susceptible to infection during their first growing season (Stone et al. 2000), but the reasons for a limited duration of needle susceptibility or the basis for resistance to infection in needles after the first growing season are not known. It is possible that susceptibility to infection of needles changes during the early period of needle development, and that there is a point in needle development where susceptibility is greatest, after which it declines. Further investigation is needed to elucidate the factors affecting susceptibility in needles.

One factor potentially affecting susceptibility to infection that is influenced by host genetics as well as cultural conditions is the timing of needle emergence from buds. Needles that emerge from buds earlier in spring are potentially exposed to inoculum longer, or perhaps may be exposed to higher levels of inoculum during a period of peak susceptibility. Seedlings in this study were monitored for phenological development and shoot development. There was a general correspondence between timing of bud break and rate of shoot emergence and infection index. A ranking of seed sources in order of rate of bud emergence and shoot elongation correlated with infection index with an r^2 of 0.64. This suggest one possible avenue for further study to attempt to identify the underlying basis for variation in susceptibility to infection.

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Effect of Climate on Infection Biology and Epidemiology of *Phaeocryptopus gaeumannii* and Swiss Needle Cast

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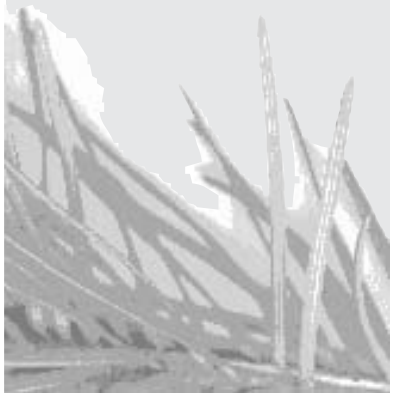
Abstract

Disease severity varies between sites affected by Swiss needle cast in the Oregon Coast Range, but within sites measures of severity have remained at similar levels over four years. Severity of disease appears not to be increasing in affected coastal Oregon sites, but in sites with severe disease it is not diminishing. Levels of *P. gaeumannii* are negatively correlated with needle retention. Disease severity is influenced by site specific factors that favor pathogen growth. Climatic factors that favor fungal infection and colonization are strongly correlated with disease severity measures. Environmental parameters that correlate well with pathogen growth and disease severity are winter degree day accumulation and spring/summer leaf wetness at 14-16 C.

Introduction

Despite periodic interest in the disease, there has been little documentation of the distribution and severity of Swiss needle cast in forests in the Pacific Northwest. Most of the published research on Swiss needle cast comes from Christmas tree plantations or forests in Europe or New Zealand where Douglas-fir is planted as an exotic species (Boyce 1940, Chastagner 1996, Hood, 1996, Hood and Kershaw 1975). Severe defoliation and growth reduction due to Swiss needle cast disease were observed in Douglas-fir plantations in Switzerland as early as 1925 (Gaeumann 1930) and subsequently reported from other European countries and eastern United States (Boyce 1940), New Zealand, and Australia (Beekhuis 1978, Hood 1996). However, factors affecting the distribution and severity of the disease in the forests of the Pacific Northwest where both Douglas-fir and the pathogen are native have received little attention. In contrast to the situation in Europe and other areas where Douglas-fir is cultivated, defoliation due to Swiss needle cast has not been documented in forests of the Pacific Northwest until recently (Hansen et al 2000).



Phaeocryptopus gaeumannii, the causal agent, is widespread in the Pacific Northwest, but until recently was considered insignificant in North American forest situations (Boyce 1940, Hood 1982). Although recent concern about Swiss needle cast near Tillamook dates from 1990 or later, there were earlier observations of the disease in the vicinity, and it is almost certain that the fungus is native to the area. Meinicke

(unpublished, cited in Boyce 1940) collected the fungus in 1938 near Otis Junction and Hebo near the center of the current area of severe symptoms, but stated that it was not causing defoliation or yellowing. In comparing the effect of *P. gaeumannii* on its host in the Northwestern U.S. to that in Europe and the eastern U.S., Boyce (1940) stated: "Within the natural range of Douglas-fir in western North America the fungus has been present for many years, although it passed unnoticed…because there the fungus is either not at all or so negligibly injurious to the host that it is easily overlooked." and later "In the Douglas-fir region of the Pacific Coast, even though the fungus is prevalent, it has caused no injury." The amount of foliage discoloration, defoliation, and growth loss observed in Douglas-fir in the Oregon Coast Range due to Swiss needle cast in recent years indicates an intensification of the disease since Boyce's observations.

The possible reasons for the widely different effects of infection by *P. gaeumannii* on Douglas-fir in Europe compared to that in the western U.S. were considered by Boyce (1940), and similarly the possible causes of the apparent change in effects of *P. gaeumannii* on Douglas-fir in the coastal region since Boyce concern us now. Boyce (1940) proposed four possibilities to account for difference in effects of *P. gaeumannii* in Europe and the Pacific Northwest, and these same possibilities have been investigated with respect to the recent epidemic. Possible causes proposed by Boyce were: 1) A new pathogenic strain of fungus, a derivative of a weak parasite or a hybrid strain; 2) A novel strain or species introduced into Europe and eastern U.S. from Asia; 3) *P. gaeumannii* is really *P. nudus* that jumped a new host; 4) *P. gaeumannii* is a nearly harmless parasite native to the western U.S. and Canada, introduced into Europe and eastern N. America where it is more pathogenic due to climatic conditions more favorable to the development of the fungus. Of these possible explanations, Boyce favored the latter: "Hence it seems not unreasonable to assume that *Adelopus gaeumannii* has changed from a harmless parasite on the Pacific Coast to a pathogen in the different climates of the northeastern United States and Europe."

Boyce's hypothesis that a climate more favorable for growth of *P. gaeumannii* primarily accounts for the SNC epidemic in Europe has survived as the most widely accepted explanation for differences in disease severity between Europe and western North America, although specific contributory climate factors have not been identified. Similarly, climate factors may be primary determinants of the current SNC epidemic in Oregon. Higher than historically normal levels of *P. gaeumannii* may have developed in areas of coastal Oregon due to recent climate change, short term variations in weather patterns influenced by ocean currents, or increased acreage of Douglas-fir plantations on sites with climate conditions particularly favorable to the development of the fungus. We have been monitoring several measures of fungal colonization and disease severity in relation to environmental and site factors to attempt to identify site climate characteristics that might be useful for prediction of disease risk and understanding environmental factors influencing fungal growth and development. The objectives of this research are to determine whether the epidemic of Swiss needle cast in Oregon is intensifying, to identify environmental factors that correlate with measures of fungal colonization and disease severity, and to understand how growth of the pathogen is influenced by climate. Additional greenhouse/ shade house experiments have been conducted concurrently to examine effect of certain environmental variables on colonization and development of *P. gaeumannii* in foliage and the relationship between colonization and needle retention.

Materials and Methods

Measurements of disease impact were made at nine monitoring plots first established in 1996. The nine plots are grouped in three clusters of three plots each in the vicinity of Tillamook, Oregon (Table 1). The plots in each cluster were placed in Douglas-fir plantations of the same age and, where possible, the same seed source. Plantations were selected to represent different elevations and distances from the ocean and exhibited a range of disease severity. One plot in each group has moderate to heavy symptoms of Swiss needle cast, and one site is classified as healthy.

The South Cluster of plots are all USDA Forest Service progeny test plantations. Ten trees of each of two families were selected for measurements in each plantation. Plots of the Tillamook Cluster were planted

with seedlings from the same bulk seed lot, from the "Boundary" seed collection area of the Coast Ranges, at about 600 m elevation. Ten trees were randomly selected for measurements in each plantation. The North Cluster included one plot planted with the Boundary source (North Fork) and two Oregon Department of Forestry progeny test plantations. Ten trees of each of two families (different from the South plots) were selected for measurements in each of the latter plantations, and ten trees were measured at North Fork.

Each study site is equipped with weather monitoring equipment. A tipping bucket rain gauge, leaf wetness sensors, and temperature sensors are maintained at each site. Symptoms of Swiss needle cast have been measured annually at all nine sites since 1999. Tree height and diameter are measured, and a visual index of crown density and transparency is made for each tree. Needle retention is determined for two lateral branches from the mid crown (fifth whorl below the terminal shoot), and foliage samples are collected for determination of infection incidence and severity for each tree. All measurements and foliage collection were done in the spring just prior to bud break.

For determination of infection incidence and severity, needles for each age class/tree were stripped from branches and combined. Fifty needles for each age class/tree were randomly selected and affixed to index cards with double-faced adhesive tape. The needles on index cards were then examined under a dissecting microscope. Incidence of infection was recorded as the proportion of 50 needles bearing pseudothecia of *P. gaeumannii*. Severity, the number of stomata occluded by pseudothecia, was determined by counting pseudothecia on ten needles. Three sections, 2.6 x 0.26 mm, of each needle were counted with the aid of a dissecting microscope. Another sample of ten randomly selected needles from each age class/tree was used for quantitative PCR analysis (Winton et al in press).

Statistical analyses of differences in needle retention, pseudothecial density, PCR value (normalized *P. gaeumannii* DNA) between sites were carried out by ANOVA with Fisher's LSD multiple comparisons procedures. Correlations between needle retention, pseudothecial densities, PCR value, and leaf wetness were carried out with SYSTAT v. 9.0.

Table 1. Characteristics of field plots.

SITE	Disease Severity	Elevation	MILES TO OCEAN/BAY	AGE (1996)	SEED SOURCE	Aspect
JUNO HILL	Severe	380	2.25	14	Boundary 1800FT	NE
STONE RD LOWER	Mild	430	14.75	14	Boundary 1800FT	SW
STONE RD UPPER	Healthy	1700	14.5	14	Boundary 1800FT	N
N FORK	Severe	160	4.75	10	Boundary 1800FT	SW
COAL CRK PROGENY	Moderate	220	5	10	1600FT & 1400FT	SE
ACEY CRK PROGENY	Healthy	670	8	10	1600FT & 1400FT	E
SALAL PROGENY	Moderate	370	4	9	1000FT	NW
CEDAR NORTH PROGENY	Mild	1500	7.5	9	1000FT	NW
LIMESTONE PROGENY	Healthy	890	12.25	9	1000FT	N

Results

Retention of two-year-old needles is a good indicator of disease severity at the nine coastal sites. The most severely diseased sites in each group (north, central, south) have the lowest retention of two-year-old needles. During the past four years, retention of two-year-old needles has varied, but there is no indication yet that needle retention at diseased sites is decreasing, or that SNC at healthy sites is intensifying (Figure 1). This is despite an incidence of SNC on one-year old foliage very close to 100% at all sites. Needle retention at the two most severely diseased sites, Juno Hill and North Fork, increased in 2001, possibly indicating a lessening of disease severity. Foliage discoloration follows a similar pattern, with Juno Hill and North Fork showing the most severe symptoms (Figure 2).

Number of pseudothecia on one- and two-year-old needles (pseudothecial density) is an indication of the degree of fungal colonization of foliage, and is inversely related to needle retention at the study sites (Figure 4). Juno Hill, the most severely diseased site has a average needle retention score of 2.8, and pseudothe-

cia occupying 53.4% of two-year-old needles and 22.8% of one-year-old needles. In contrast, sites with low or moderate disease such as Upper Stone and Acey Creek have over 80% of second year foliage, and about 15% of stomata occluded in two-year-old needles and less than 5% for one-year old needles (Figure 3). A strong correlation between pathogen colonization and needle retention was also supported by studies on potted seedlings. In potted seedlings that were exposed to varying levels of inoculum in May-June 1999 and then assessed for pseudothecial density and quantitative PCR in the spring of 2000, and one-year-old needle retention in November, 2000, needle retention was strongly correlated with measures of pathogen colonization (Figures 5, 6).

Pseudothecial densities on two-year-old needles also varied over the past three years, but variation is generally greater among sites than between years within sites (Figure 7). Pseudothecial densities in 2001 were lower than the preceeding year for the north and middle groups of sites, but appear to have been steadily increasing in the southern set of sites (Figure 7). Sites with the greatest numbers of pseudothecia on two-year-old needles, Juno Hill and North Fork, also have the most severe disease symptoms (Figs. 1, 2). Pseudo-thecial densities on two-year-old needles (1998 needles sampled in April 2000) for the two most severely diseased study sites, Juno Hill and North Fork, were statistically equivalent but greater than all other sites at $p < 0.001$. Pseudothecial densities of one-year-old needles

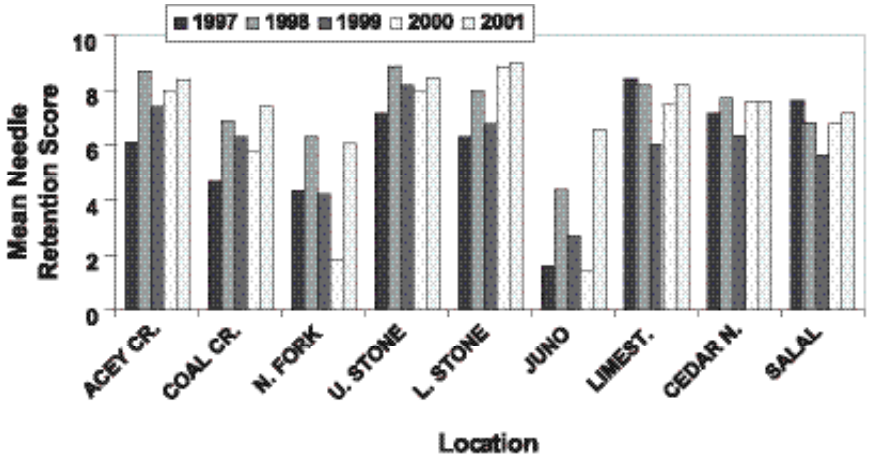


Figure 1. Yearly patterns of needle retention in nine coastal Oregon SNC study sites 1997-2001.

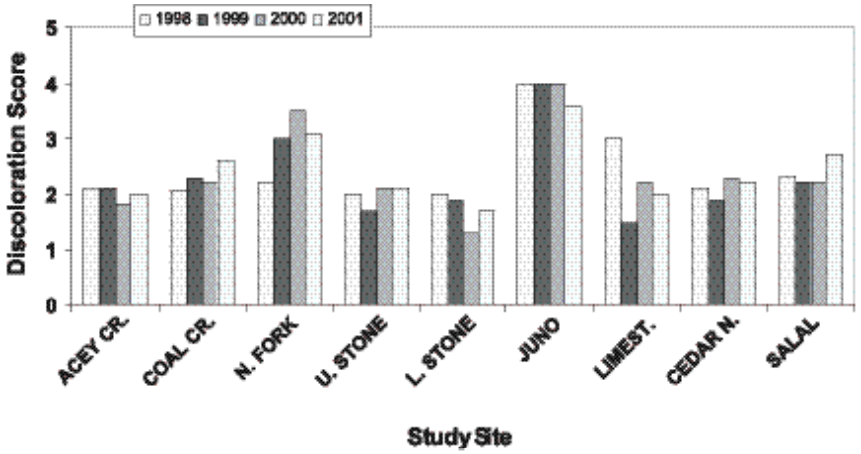


Figure 2. Foliage discoloration in nine coastal Oregon SNC study sites 1998-2001.

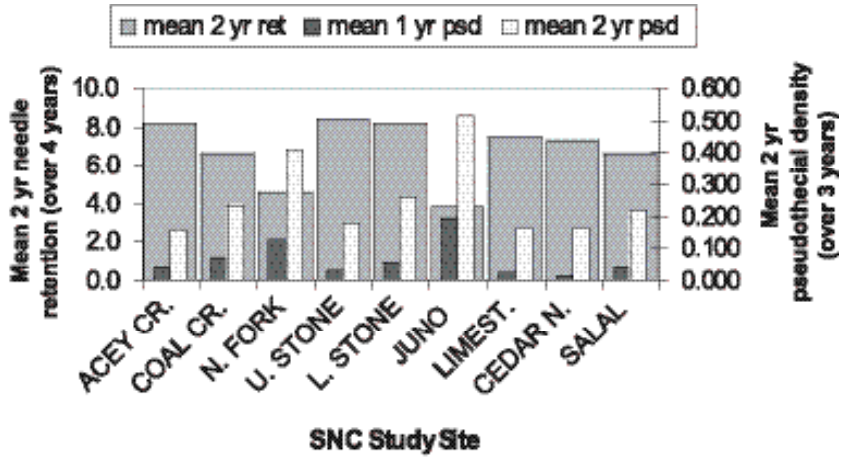


Figure 3. Mean needle retention over five years (1997-2001) and pseudothecial density over three years (1999-2001) for nine coastal Oregon SNC study sites.

(1999 needles sampled in April 2000) were also highest for Juno and North Fork but were significantly different from each other. In general, density of pseudo-thecia on one-year-old needles closely parallels the values for two- year-old needles within each site (Figure 3).

Pseudothecial densities on one- and two-year needles within sites were very highly correlated (Pearson coefficient = 0.952, p , 0.001), as expected. Retention of two-year-old needles was highly correlated with pseudothecial densities on two-year-old needles (Pearson coefficient = 0.912, P < .01), and with pseudothecial densities on one-year-old needles (Pearson coefficient = 0.869, p < 0.05). PCR analysis of one- and two-year-old foliage agreed well with the pseudothecial density measurements (Table 2, Figures 8, 9). For two-year-old foliage, the Juno site had the highest colonization levels detected by quantitative PCR, followed by North Fork and Salal in one statistical group, with the remaining six sites not statistically different. Quantitative PCR values were also correlated with retention of two-year old-needles (Figure 10).

Because infection levels and disease severity within sites appear to have remained at relatively constant levels over time, and because infection levels remained similar within sites from year to year, environmental variables that differ across sites and that might affect fungal growth were investigated. Free surface moisture is necessary for infection by most foliar plant pathogens, and in general higher infection levels are associated with prolonged periods of free moisture.

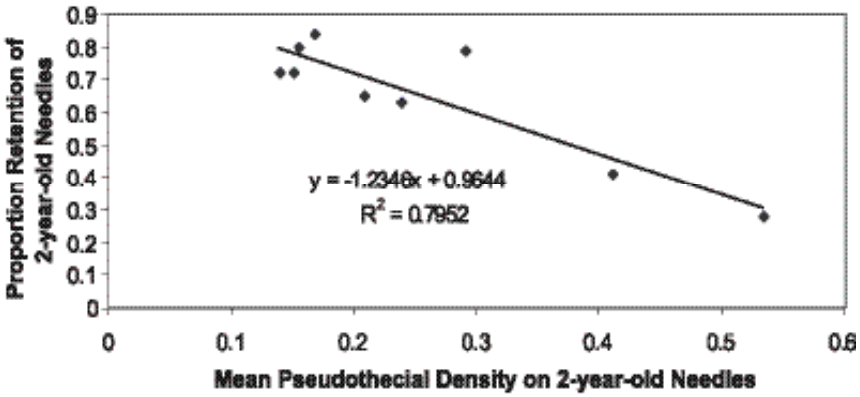


Figure 4. The relationship between retention of two-year-old foliage and pseudothecial density. Seedlings were exposed to inoculum for varying periods May-June 1999 and returned to the OSU Botany Field Lab for incubation. Pseudothecial density and needle retention were determined on the 1999 shoots in spring 2001.

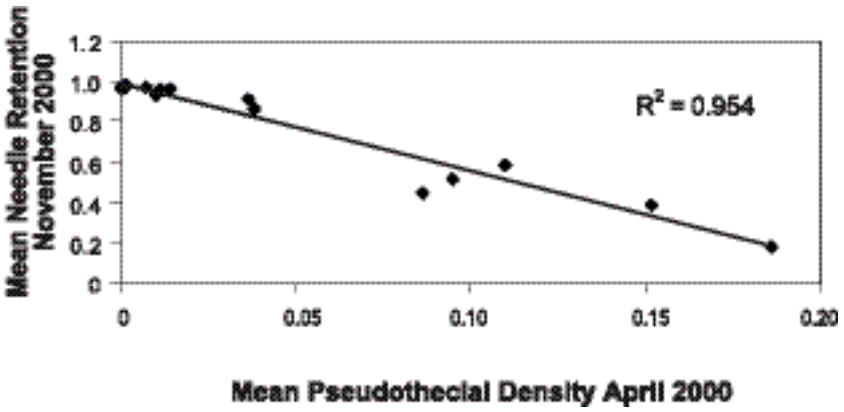


Figure 5. Relationship between pseudothecial density in April 2000 and retention of one-year-old needles in November 2000. Seedlings were exposed to inoculum for varying periods May-June 1999 and returned to the OSU Botany Field Lab for incubation.

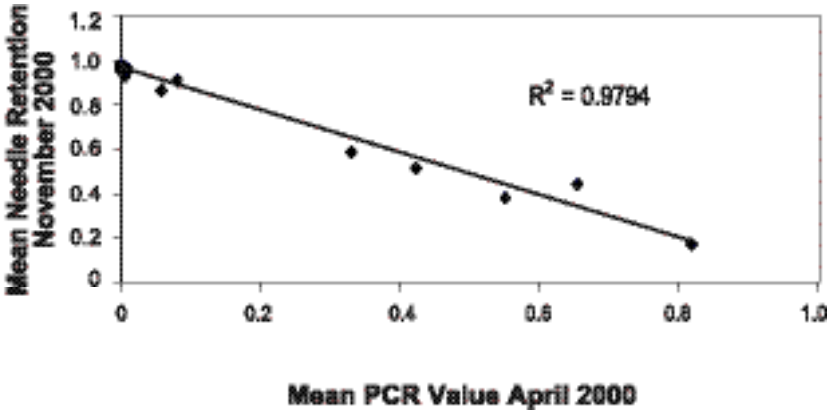


Figure 6. Relationship between P. gaeumannii *biomass (QPCR) in April 2000 and retention of one-year old needles in November 2000. Seedlings were exposed to inoculum for varying periods May-June 1999 and returned to the OSU Botany Field Lab for incubation.*

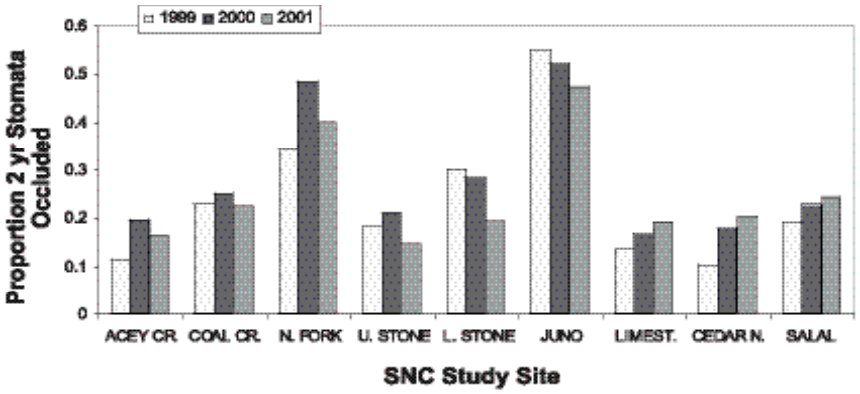


Figure 7. Abundance of pseudothecia on foliage for three years at nine coastal Oregon SNC study sites.

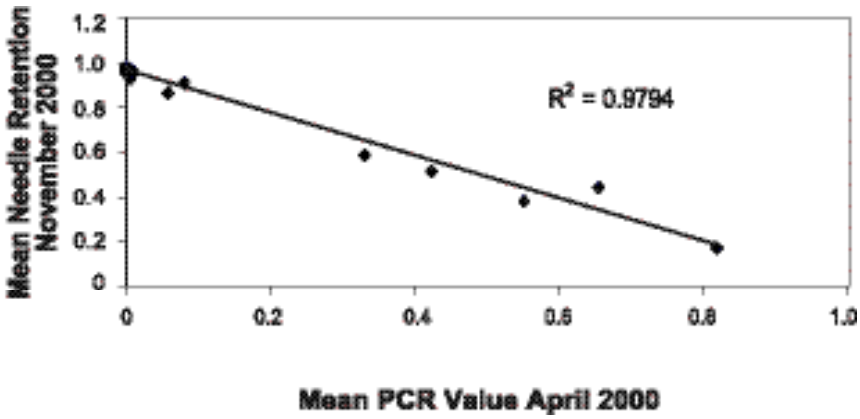


Figure 8. Quantitative PCR analysis of Douglas-fir foliage from nine coastal Oregon SNC study sites. Foliage was collected and analyzed in April, 2000. Needles were one (1999) or two (1998) years old.

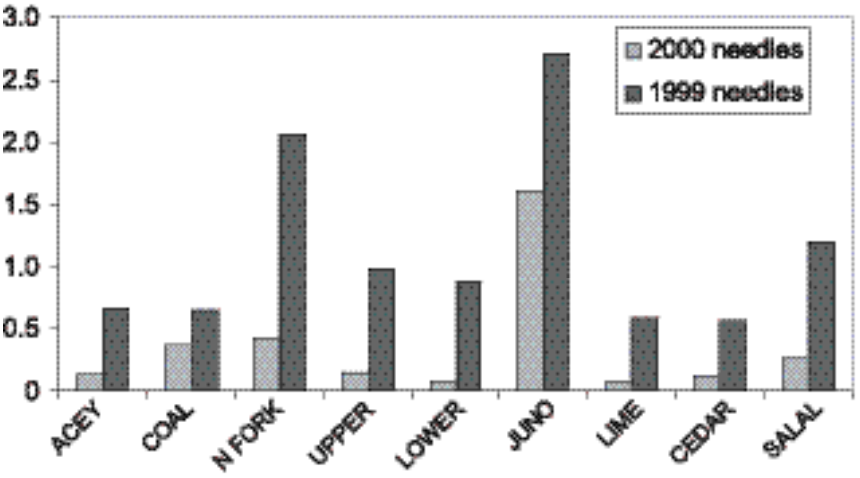


Figure 9. Quantitative PCR analysis of Douglas-fir foliage from nine coastal Oregon SNC study sites. Foliage was collected and analyzed in April, 2001. Needles were one (2000) or two (1999) years old.

The optimal temperature range and optimum period for fungal growth also should be considered. Several combinations of temperature range, threshold leaf wetness values, and season were tested for separation of sites by disease severity and pathogen abundance. Two sets of parameters provided good correlations with amounts of fungal colonization at sites: leaf wetness hours at 14-16 C between May – Oct (Figure 11) and winter degree day accumulation 6 – 26 C (Figure 12).

Discussion

There is a strong correlation between levels of *Phaeocryptopus gaeumannii* and defoliation and discoloration of Douglas-fir in coastal plantations in Oregon. The number of pseudothecia on one- and two-year-old foliage is highly correlated with total needle retention at sites. Regardless of age, needles are abscised when more than approximately 50% of stomata are occupied by pseudothecia (Hansen et al. 2000), a consequence of impaired gas exchange (Manter et al 2000). Our evidence points to Swiss needle cast as the primary cause of needle loss and not a secondary colonist of foliage weakened by another agent. Consistently greater needle retention in foliage sprayed with chlorothalonil indicates the involvement of a fungal foliar pathogen in the defoliation of Douglas-fir trees on the Oregon coast. *Phaeocryptopus gaeumannii* is the only foliar pathogen present on the trees before treatment, and is the only pathogen that is abundant throughout the area of the epidemic (Hansen et al 2000).

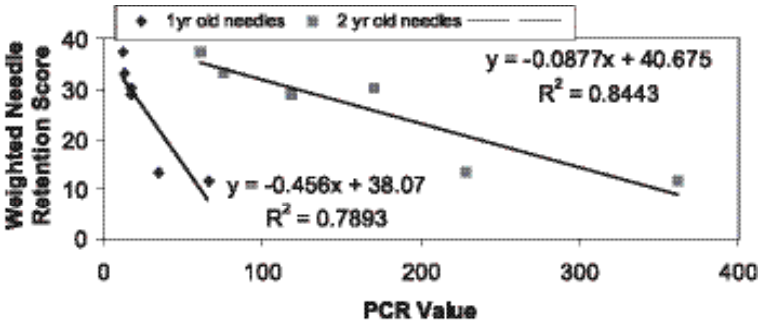


Figure 10. Relationship between colonization of foliage by P. gaeumannii *and needle retention. Amount of* P. gaeumannii *in foliage determined by quantitative PCR.*

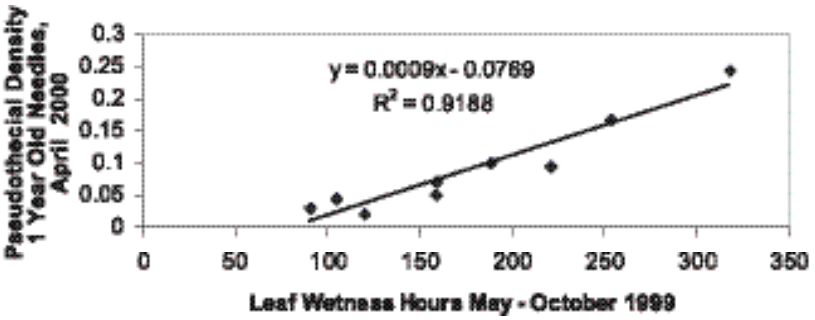


Figure 11. Relationship between cumulative leaf wetness at 14 – 16 C between May and October, 1999 and abundance of pseudothecia on one-year-old needles for nine coastal Oregon SNC study sites.

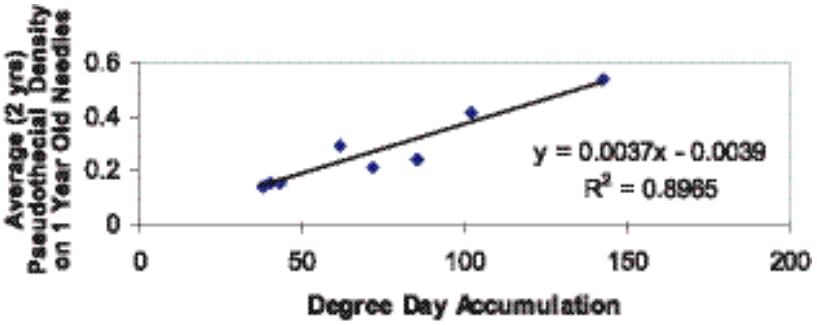


Figure 12. Relationship between cumulative winter (December – February) degree days at 6 – 26 C and abundance of pseudothecia on one-year-old needles for nine coastal Oregon SNC study sites.

A puzzling aspect of the current Swiss needle cast epidemic is concerns why the disease apparently intensified since about 1990. Periodic observations indicate that the fungus has long been present in the area, and periodic disease outbreaks have been noted (Russell 1981, Hansen et al 2000). Measurement of disease symptoms of Swiss needle cast over the past four years do not indicate a rapidly developing epidemic, and suggest that at the southern coastal sites disease severity is increasing gradually while remaining relatively constant in the northern and central sets of sites. Sites with high disease have remained high, while sites with moderate or low disease have not changed during the past four years. Although the disease may be becoming more serious further inland from the coast, levels of infection and defoliation have remained relatively constant over four years in individual coastal study sites. Disease severity among sites is variable however, and within each group (north, south, central) a range of disease severity can be seen that has remained constant within sites over three years. Because the same seed source was used within each group of sites, consistent differences in disease severity among sites suggests environmental or other site related factors are involved.

Factors that may be important in the current outbreak of Swiss needle cast are increased inoculum levels, increased acreage of Douglas-fir plantations in the coastal area and subtle climatic differences between sites that favor infection and colonization by *P. gaeumannii*. In 1997 the Oregon Department of Forestry (unpublished data) investigated the history of 76,970 ha of Douglas-fir plantations 10 – 30 years old growing within 29 km of the north Oregon Coast. About 31% of these plantations had been established on sites where hemlock and spruce had dominated the previous stand. Only 20% were on sites dominated by Douglas-fir in the previous rotation. The remaining areas were mostly alder stands that had been converted to Douglas-fir. Although historical records are scant, this at least suggests that Douglas-fir is more abundant in the coastal forests than earlier this century. Increased levels of Douglas-fir in the coastal area may be a contributing factor to the current SNC problem. If recent conditions have been favorable for development of the pathogen, then higher than normal levels of inoculum

may have been produced in recent years.

Much of the land that has been converted to Douglas-fir plantations in recent decades, and where most severely affected plantations are located, lies in the *Picea sitchensis* vegetation zone, a narrow strip of coastal forest characterized by elevations generally below 150 m, proximity to the ocean, and a moderate climate. Although Douglas-fir is the natural seral dominant in the *Tsuga heterophylla* Zone, which borders the *Picea sitchensis* Zone to the east, its occurrence in the *Picea sitchensis* Zone is more sporadic, and normally it occurs in mixtures of spruce and hemlock, not as pure stands (Franklin and Dyrness 1973). An increase in the proportion of Douglas-fir in recent decades, its concentration in pure stands, and favorable climatic conditions may have enabled *P. gaeumannii* to increase above historically normal levels in coastal forests, leading to increased disease pressure. Under this increased inoculum pressure, even a naturally tolerant host population may be adversely affected.

Subtle climatic differences that are primarily responsible for the different vegetation composition of the *P. sitchensis* and *T. heterophylla* zones, also are likely important factors in disease severity. Hood (1982) found higher levels of *P. gaeumannii* in southern British Columbia and western Washington in coastal forests of Vancouver Island and the Olympic Peninsula, with lower levels in the rain shadow of eastern Vancouver Island and the interior. In our plot clusters, disease symptoms are more severe and fungal colonization greatest in sites with low elevation near the coast. At slightly higher elevations and further inland, plantations of the same age and seed source have milder symptoms of disease and needle retention of 3 to 4 years on average. The fungus is still abundant but predominantly on the older needles. Environmental differences between such nearby sites are subtle but perhaps significant. Temperatures are milder and annual rainfall is actually lower closer to the coast in the *Picea sitchensis* Zone than it is at higher elevations in the Coast Range. Hood (1982) found a significant correlation between May-July rainfall and percentage of infected needles. At our sites, measurement of leaf wetness at a temperature range where *P. gaeumannii* should be physiologically active (14-16 C) was related to differences in disease severity among sites, and the degree day accumulation 6-26 C from Dec-Feb was strongly correlated with needle colonization by *P. gaeumannii*. Mild winter temperatures together with spring-summer leaf wetness at 14-16 C therefore appear to be a possible predictors of SNC severity and could be used as criteria for evaluating suitability of Coast Range sites for Douglas-fir. The finding that temperature during the winter months may be an important determinant of *P. gaeumannii* growth is supported by results of Manter et al (2001, this volume).

Genetic composition of the Douglas-fir plantations may also be important. In Douglas-fir progeny tests in British Columbia (Hood 1982) seedling families originating from drier areas east of the Coast Ranges were more susceptible to SNC than families collected from wet forests near the coast. In many cases in the Tillamook area, seed used in low elevation coastal plantations originated from higher elevations in the Coast Ranges. The most severe disease symptoms in our study sites are at the Juno Hill and North Fork sites. Both sites were planted with seed designated Boundary 1800 ft, but both sites are below 400 ft elevation. It might be expected that trees adapted to climates less favorable to the fungus would have less tolerance to infection than trees from areas where conditions favor colonization and evolution of genetic resistance would have a high selective benefit. Evidence of genetic variation in Douglas-fir from Coast Range provenances is reported by Stone et al. (2001 this

Table 2. PCR analysis of foliage from nine coastal study sites. Letters following values in rows are not different at p<0.01. Results of spring 2000 and spring 2001 samples.

PCR	Acey	Coal Creek	North Fork	Upper Stone	Lower Stone	Juno	Lime-stone	Cedar	Salal
2000									
AC 98	.096 a	.189 a	.641 c	.185 a	.187 a	1.615 b	.188 a	.119 a	.511 c
AC 99	.032 a	.085 a	.354 c	.042 a	.101 ab	.440 c	.054 a	.038 a	.196 b
2001									
AC 99	.666 c	.645 c	2.069 a	.983 b	.870 bc	2.707 a	.579 c	.567 c	1.200 b
AC 00	.131 c	.364 bc	.413 b	.139 c	.082 c	1.616 a	.076 c	.110 c	.258 bc

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Nutritional Imbalance as a Predisposing Factor in Swiss Needle Cast Disease:

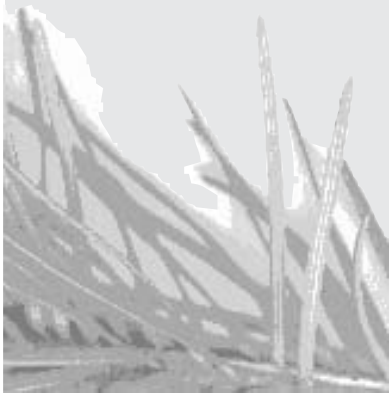
Part I. The Role of Free Amino Acids
Part II. A Field Test With Fertilized Seedlings

Cathy L. Rose, Kathleen Kavanagh, Richard Waring, and Dan Manter

Introduction

Nutrition can be an important factor in tree susceptibility to certain fungal pathogens. In particular, high foliar N has been implicated as a predisposing factor in fungal infection of conifer needles (Turner and Lambert 1978, Carroll 1986, Schulze et al. 1989). Conifers store surplus N primarily in the form of free amino acids (FAA), consequently trees subject to high N availability readily accumulate FAA in needles (Runge 1983, Larsson et al. 1986, Nasholm and Ericsson 1990, Shainsky and Rose 1994). Substrates high in FAA support rapid growth in diverse fungal taxa, including many foliar fungi (Cochrane 1963, Kelly and Lambert 1972, Turner and Lambert 1978, Jennings 1995). Consequently, nitrogen nutrition may be an important factor in the management of forest susceptibility to fungal pathogens.

The current outbreak of Swiss needle cast caused by *Phaeocryptopus gaeumannii* on Douglas-fir in western Oregon appears to be associated with elevated foliar concentrations of total N and FAAs, and depleted levels of other elements, including Ca (Maguire et al. 2000, Rose et al. 2000). Recent field studies in the Coast Range of Oregon and Washington provide further evidence of nutritional imbalance in this region (Miller et al. 1999, Cromack et al. 1999, Maguire et al. 2000). Potential causes of high N availability in the Pacific Northwest have received little attention, but coastal forests of the Pacific Northwest may be especially prone to imbalance as they tend to accumulate N over much longer time periods than other ecosystems (Edmonds et al. 1995, Blew and Edmonds 1996). Vegetation processes favoring N fixation and retention have been cited as driving factors in N enrichment (Johnson and Lindbergh 1992, Aber et al. 1998). Moreover, soil interactions may exacerbate other nutritional imbalances, resulting in low availability of other nutrients, such as base cations and S (Turner, 1977, Turner and Lambert 1986, Fenn et al. 1998). With widespread N enrichment, nutritional imbalances are becoming increasingly common in forest ecosystems, even on inherently fertile soils (Schulze et al. 1989, Aber et al. 1998, Yanai et al. 1999, Magill et al. 2000).



Part I of this study had two main objectives: (a) to determine if there is an association between the severity of Swiss needle cast disease and foliar concentrations of N and free amino acids, and (b) to examine whether foliar nutrient imbalances respond to foliar applications of fungicides, or S fertilizer. The objective in Part II of this study was to test whether fungal growth in needles (quantified by PCR methods) responds to foliar concentrations of total N and FAA in potted 1-0 seedlings grown under a wide range N supply. Findings from Part I of this study have been presented earlier (Rose et al. 2000). In this report, we present the final data set collected in Part I and summarize recent progress on Part II of this study.

Methods

Methods for Part I of this study have been described previously (Rose et al. 2000). Douglas-fir stands from 12-20 years old were sampled at four locations in western Oregon to reflect a variety of forest conditions (Table 1) : 1) diseased trees exhibiting a range of disease severity (visual assessment), 2) upper crowns of heavily-infected trees treated with or without Bravo fungicide, 3) individual branches of heavily-infected trees treated with or without Bravo fungicide, and 4) heavily-infected trees treated with or without Thiolux S fertilizer. Samples collected from these sites were analyzed for free amino acids at three intervals: pre-budbreak, post-budbreak, and dormant periods. A subset of these samples were also analyzed for total N and S, other macronutrients, and sulfate-S.

In Part II of this study, 60 4-0 Douglas-fir seedlings were re-potted in October 2000 for application of different N fertilizer regimes. To hasten induction of N deficiency in low N treatments, and to facilitate transport of seedlings to and from field inoculation sites, these seedlings were later discarded in favor of smaller 1-0 Douglas-fir. In January 2001, 120 of the 1-0 seedlings were re-potted and maintained outdoors under 50% shade at the OSU botany farm.

Seedlings for this experiment were partitioned into 4 blocks and five treatments, with six seedlings per block. Fertilizer solutions were formulated to create a wide range of N availability based on recommendations provided in Walker and Gessel (1991). The fertilizer treatments consisted of different levels of N supply in combination with complete and balanced macronutrients (Table 2). The seedlings were fertilized weekly with liquid solutions from January through May, 2001. At the end of May (the pre-inoculation sample), samples of current-year needles were collected for determination of total N content, free amino acids, and fungal biomass by PCR. Samples included 25 current-year needles from single branches on three seedlings per 4 blocks and 5 treatments, for a total of 60 samples.

After June 1, 2001, the frequency of fertilizer application was reduced to once every two weeks in order to achieve a more rapid reduction of foliar N in low N treatments. Seedlings were transported to a naturally-infected Douglas-fir plantation (Salal Plot, Cloverdale, OR; Hansen et al. 2000) during the month of June, 2001 for field inoculation of needle. After inoculation, seedlings were returned to ambient conditions at the OSU Botany Farm for continued growth and fertilizer application. Additional sample collections were scheduled in August and October, 2001. Concen-

Table 1. Description of foliar treatments and sampling protocol for each study site sampled in Part 1 of this study. Needles were sampled to document concentrations of free amino acid in coastal Douglas-fir saplings infected with Phaeocryptopus gaeumannii.

Site	Location	Foliar samples taken from:
Juno Hill	Near Tillamook OR, Juno Hill.	1) 3 unsprayed control branches from 3 trees. 2) 3 branches from the same 3 trees that had surface applications of Bravo. Applications by Alan Kanaskie. Not sure of the rate. All branches were sprayed at bud break in 1995-1998.
Toledo	Near Toledo OR, So. Drake.	1) 3 unsprayed control trees. 2) 3 trees with surface application of sulfur (Thiolux) at 2 ounces per tree. Applications were made on June 8, June 25 and July 10th 1999.
Beaver Cr.	Beaver OR, Siuslaw NF	1) 3 unsprayed control trees and 2) 3 trees with surface application of chlorothalonil (Bravo 720, rate = 66 ml / 3.78 L, applied until run-off. Applications were made 1998, 1999 and 2000 at budbreak and one month later.
Coal Cr.	Near Nehalem OR, ODF	1) 3 trees with low SNC disease symptoms (retain 3 years of foliage) and 2) 3 trees with high SNC disease (retain only 1.2 years of foliage). No foliar applications have been made to these trees.

Table 2. In Part 2 of this study, five fertilizer formulations were used to produce a wide range of N supply to 1-0 Douglas-fir seedlings. Fertilizer formulations were based on recommendations in Walker and Gessel (1991). Nutrient are in units of millimoles (mM).

Target Values for Nutrient Supply						
Fertilizer Treatment	N	P	K	Ca	S	N Supply
1	25	40	80	40	6	deficient N
2	50	40	80	40	6	deficient N
3	100	40	80	40	6	*optimum growth
4	200	40	80	40	6	luxury N
5	400	40	80	40	6	luxury N

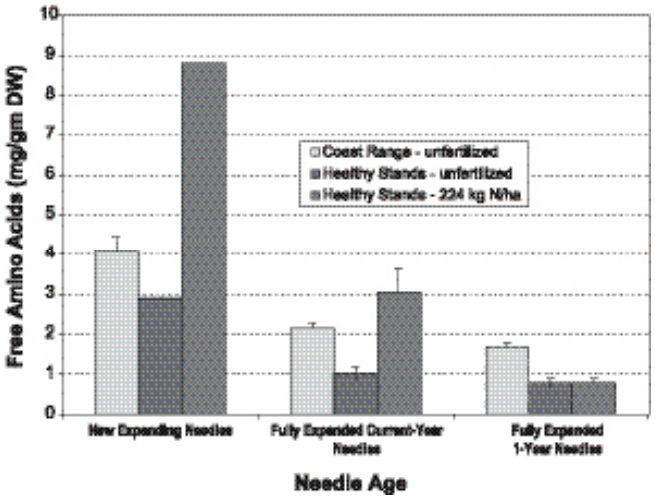


Figure 1. Comparison of foliar amino acids in coastal Douglas-fir stands to published values for 15 to 20-year-old stands.

trations of total N and other elements were analyzed by mass spectrometry within a month of the sampling date. Free amino acids are scheduled for analysis during October to November, 2001, and PCR analyses will be conducted during winter 2001-2002.

Results and Discussion

New foliage analyses have been completed for total N, and sulfur/sulfate, and other macronutrients since results in Part I of this study were last reported (Rose et al. 2000), Results of Phase I are listed below:

1. FAA concentrations were high overall, compared to published values for Douglas-fir
2. % N values in needles were high (at or above 1.5%) at most sites, especially in the post-budbreak period (Figure 3).
3. % N values were higher in trees exhibiting low disease severity;

   but FAA values did not vary with disease severity (Figure 2, 3).
4. The ratio of FAA/TN was higher in current-year needles of trees exhibiting high disease severity compared to low disease severity (Figure 4).

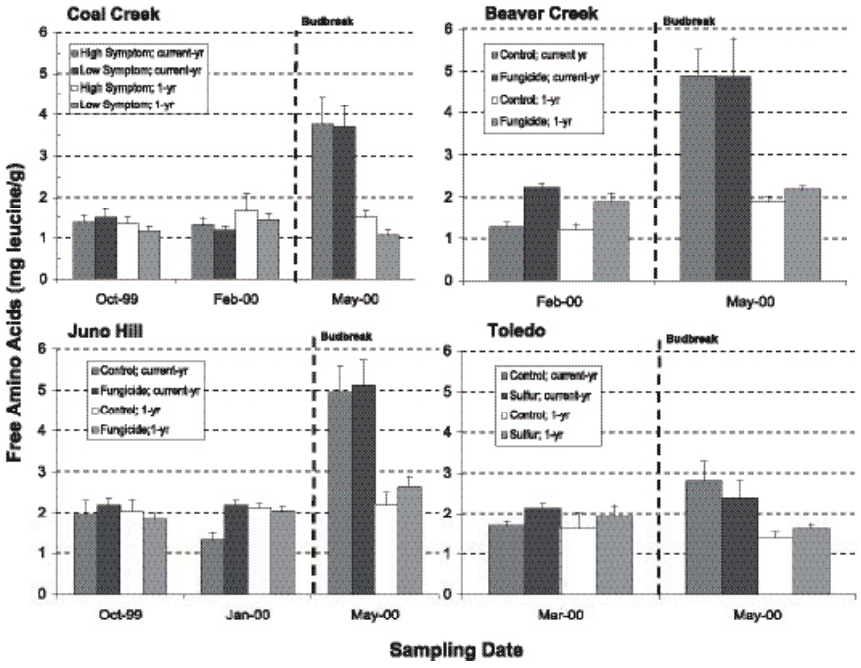


Figure 2. Foliar FAA in Douglas-fir at four sites in the Oregon Coast Range. See Table 1 for description of sites.

5. Sulfur was not a limiting nutrient at any of our sites. Rather, responses of foliage chemistry to S application were consistent with effects of a fungicidal agent (Figure 5, 6).
6. Application of Bravo fungicide was associated with an increase in foliar N and FAA and FAA:TN ratios (Figure 2,3,4).

Foliar nitrogen values were high overall and reached an average 1.5 % N (range = 0.5-2.1%) during at least one of the sampling periods for both current-year and 1-year-old

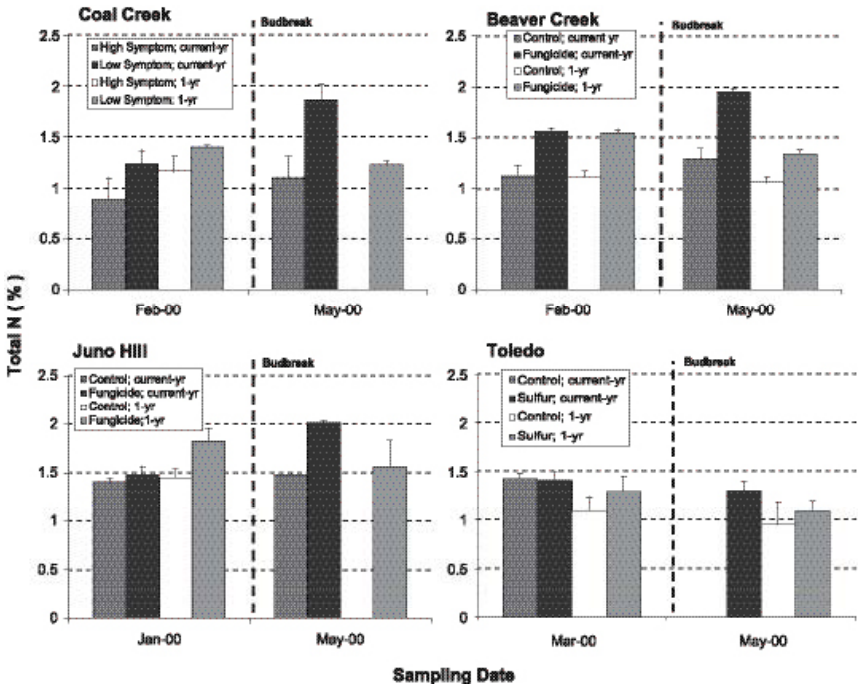


Figure 3. Foliar % total N values in Douglas-fir at four sites in the Oregon Coast Range. See Table 1 for description of sites.

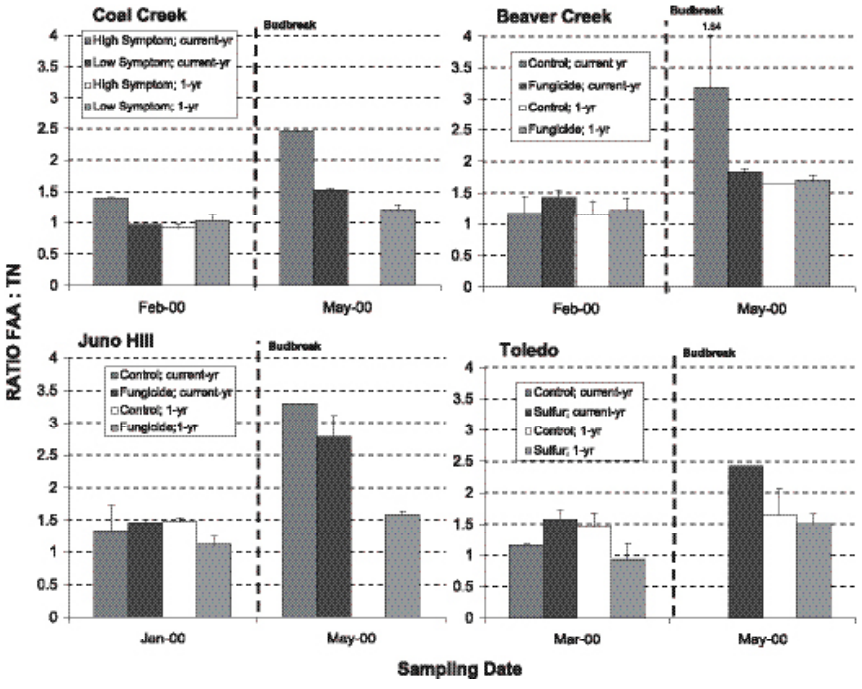


Figure 4. Foliar FAA : TN ratios in Douglas-fir at four sites in the Oregon Coast Range. See Table 1 for description of sites.

needles (Figure 3). Information from both pristine and N-enriched sites suggests that needle N values above a threshold of 1.4-1.5 % N reflect surplus N uptake in conifers (Brix 1981, Schulze et al. 1989, Katzel and Loffler 1997). Needle age classes did not show a 10-20% decline in %N from current year to 1-year-old needles as expected in healthy trees. This pattern too, is consistent with symptoms of high N supply. Evidence of high N availability at our study sites is found in elevated overall values of free amino acids compared to published values for Douglas-fir saplings (van den Driessche and Webber 1977, Brix 1981) (Figure 1). Amino acid concentrations at some of our sites approached very high values documented in young fertilized seedlings (Margolis and Waring 1986, Shainsky and Rose 1994).

Based on findings in Part I of this study, we conclude that high %N and FAA levels in foliage are probable underlying factors in the current outbreak of Swiss needle cast disease. The fact that foliar application of Bravo fungicide to whole crowns (and to a lesser degree in individual branches) caused an <u>increase rather than a decrease</u> in foliar N and FAA is consistent with the premise that *P. gaeumannii* metabolizes foliar amino acids (see Beaver Creek and Juno Hill). Elevated values of total N and FAA in fungicide-treated trees may signify pronounced nutritional imbalances that initially predisposed trees to this epidemic. The higher %N and lower FAA : TN ratio in trees with low disease severity, compared to trees with high disease severity at Coal Creek supports this interpreta-

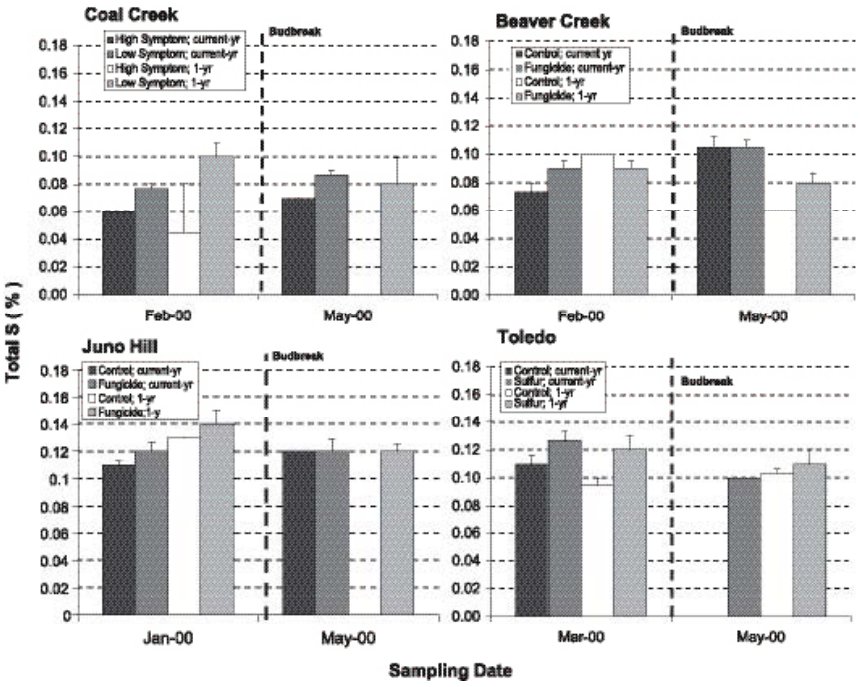


Figure 5. Foliar % total S values in Douglas-fir at four sites in the Oregon Coast Range. See Table 1 for description of sites.

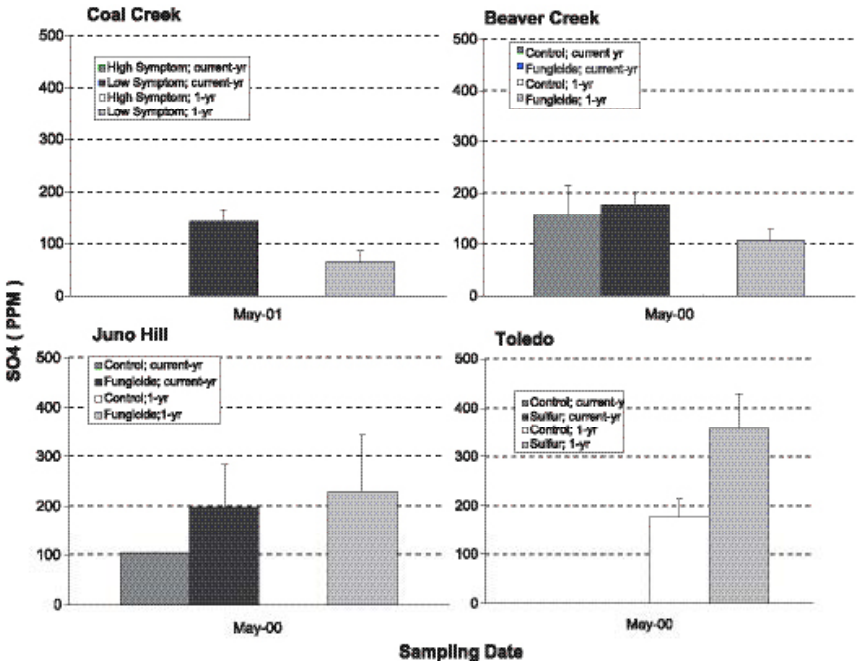


Figure 6. Foliar sulfate- S values in Douglas-fir at four sites in the Oregon Coast Range. See Table 1 for description of sites.

tion. In addition, we conclude from these data that sulfur does not appear to be a limiting nutrient at any of the sites we sampled, based on findings for % total S and sulfate-S in foliage analyses, and by the lack of a pronounced response to S application at the Toledo site (Figure 2-6).

In Part II of this study, a considerable range in seedling N nutrition has been attained after the first season of fertilizer application (Figure 7). Seedling growth and foliage color are beginning to exhibit symptoms of a wide range of N nutrition (Figure 8). Data on foliar FAA and fungal biomass will be collected and analyzed during October –December 2001. We expect seedling nutrition, growth, biochemistry, and fungal infection to diverge sharply across treatments during the second growing season. Data analysis will focus on quantifying differences in fungal growth in relation to seedling nutrition.

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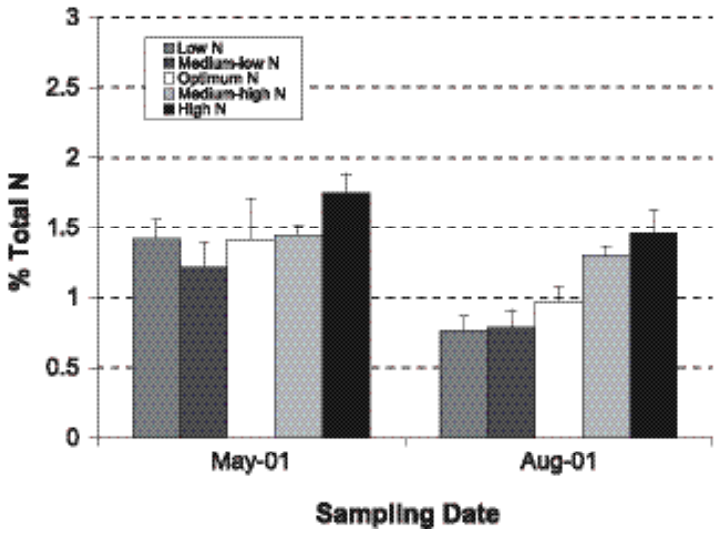

Figure 7. Foliar % total N values in 1-0 Douglas-fir seedlings treated with five levels of N supply in a balanced nutrient solution. Sampling dates are prior to inoculation in early June 2001, and in mid-August.

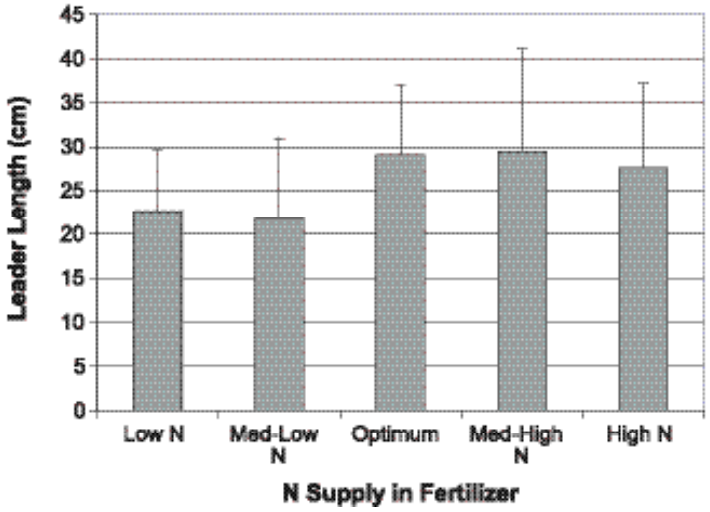

Figure 8. Leader growth in 1-0 Douglas-fir seedlings treated with five levels of N supply in a balanced nutrient solution. Sampling date is early October, 2001, after one field season.

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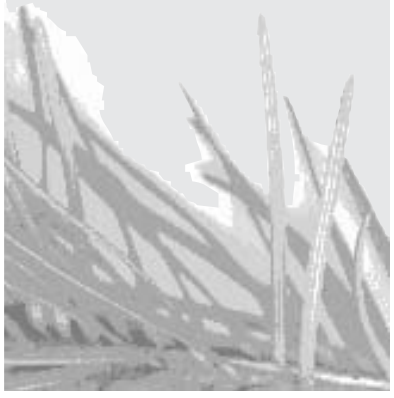
Tree responses to experimental alteration of nutrient availability across a gradient in Swiss Needle Cast Severity

Doug Maguire, Dick Waring, Kermit Cromack, and Jim Boyle

Basic research in forest nutrition has demonstrated that imbalances in plant nutrients resulting from differential availability can dramatically alter the health and productivity of trees, often through premature loss of foliage. Numerous hypotheses about the interaction between *Phaeocryptopus gaeumannii* and tree nutrition have been proposed, and limited data collected during the past two years suggest that nutrient dynamics may play a role in SNC. Work during 1999-2000 focused on soil and foliage sampling from a set of plots in the Growth Impact and Precommercial Thinning studies. Results indicated that high sulfur and nitrogen and low calcium were associated with poor needle retention. Nutrition work this year was directed at collecting foliage and soil samples from plots that have received a variety of fertilizer and/or thinning treatments. The goal of the nutrition work has been to move closer to identification of mechanisms linking SNC severity to nutritional status, and ultimately to development of operational treatments to mitigate possible imbalances, if any, that may predispose Douglas-fir to SNC. The specific objective this year was to test the hypothesis that foliar chemistry and SNC severity respond to experimental manipulations of nutrient availability. Tests of specific treatments were based on chemical analyses of foliage and soil collected on plots that have received various fertilizer and thinning treatments.

Methods

Foliage and soil samples were collected from numerous sets of silvicultural experiments that have been installed by various cooperators. A balanced fertilizer study initiated in 1996 provided most of the sites for sampling. This experiment entailed six basic treatments imposed on pairs of young Douglas-fir trees in a generalized random block design. Treatments were replicated 10 times at each site or block, and the experimental unit was a square 33x33 or 53x53-ft plot centered on a pair of measure trees. The treatments included: 1) NPK (low K); 2) NPK (high K); 3) NPK (low K) + a suite of other nutrients; 4) NPK (high K) + a suite of other nutrients; 5) N only; and 6) control (Table 1). Any one site received only four treatments, with severely impacted SNC sites receiving the high K treatments (2 and 4) and other sites receiving the low K treatments (1 and 3). High potassium treatments received 250



lbs/ac and low potassium treatments received 110 lbs/ac (Table 1).

Five trees were selected randomly from each treatment at each of 16 sites during later January and February, 2001. Depending on the site, either four or five years had lapsed since treatment. Samples of 2000 foliage were collected from the fifth whorl down from the tip of the tree, and soil samples were collected at a random distance and azimuth from the selected tree. All foliage and soil from a given treatment at a given site were combined into a composite sample. Samples were submitted to the OSU Central Analytical Laboratory for chemical analysis (Table 2). A small amount of each sample was sent separately to Jeff Stone's lab for PCR assessment of *Phaeocryptopus gauemannii* biomass.

Four primary analyses have been performed to date: 1) principal components analysis of the foliar chemistry data; 2) set of ANOVAs on individual foliage constituents; 3) MANOVA on vectors of foliage constituents; and 4) regression of fungal biomass on chemical constituents in foliage.

Table 1. Treatments in Balanced Fertilizer Study sampled for soil and foliage in 2001.

Treatment	Fertilizer and rates in lbs/ac
1	N (200), P (60), K (110)
2	N (200), P (60), K (250)
3	N (200), P (60),K (110), Mg (50), S (80), B (2), Cu (10), Fe (5), Zn (15), Ca (50)
4	N (200), P (60),K (250), Mg (50), S (80), B (2), Cu (10), Fe (5), Zn (15), Ca (50)
5	N (200)
6	Control

Table 2. Chemical analysis of soils and foliage from Balanced Fertilizer Study, including results on individual ANOVAs for treatement effect .

Elements/ cations/anions	Foliage		Soil	
	Units	p-value	Units	p-value
P	%	**0.056**	ppm	**0.01**
K	%	0.11	ppm	**0.0002**
S	%	>0.2	%	<0.2
Ca	%	>0.2	meq/100g	<0.2
Mg	%	>0.2	meq/100g	<0.2
Mn	ppm	**0.018**	-	
Fe	ppm	0.158	ppm	<0.2
Cu	ppm	>0.2	-	
B	ppm	**<0.001**	-	
Zn	ppm	>0.2	-	
Al	ppm	0.146	-	
SO_4	ppm	**0.041**	-	
SO_4-S	ppm	**0.042**	-	
C	%	0.059	%	<0.2
N	%	>0.2	%	<0.2
NH4	-		ppm	<0.2
NO3	-		ppm	<0.2

Results and Discussion

The principal components analysis showed little separation among the various treatments (Fig. 1). This result was consistent with results from the MANOVA (p=0.99). Some single elements were significantly influenced by treatment, particularly boron and manganese, as was the anion SO_4. In the case of boron, the NPK+ treatment exhibited a higher concentration than the other three treatments (control, N, NPK), a result that may not be too surprising given that boron was applied in the NPK+ treatment. Manganese concentration was higher in the control treatment than in the N and NPK+ treatments, and SO_4 was significantly higher in the control than in N. Interpretation of these results must take into account the fact that 4 or 5 years have elapsed since treatment, so manganese, for example, may have been "diluted" by any increased growth rate that may have been evoked by some of the fertilizer treatments. With respect to soils, only potassium showed any significant relationship to treatments (Table 2). Again not surprisingly, potassium concentration was higher in soils receiving the NPK and NPK+ treatments than in either the N or control treatments.

Analysis of variance indicated no treatment effect on fungal biomass as assayed by PCR (p=0.74). However, a number of significant relationships emerged when fungal biomass was regressed on variables describing foliar chemistry. The results varied among different forms of the response variable, particularly with respect to untransformed vs. logarithmically transformed variables, and with respect to raw estimates of the DNA ratio (pg fungal DNA/ng Douglas-fir DNA) vs. these estimates standardized to Juno Hill. Up to approximately 66% of the variation in the unstandardized fungal assays could be explained by foliar chemistry, but only about 52% of the variation in standardized assays was explained. The results obtained are summarized by the following best models for the four possible response variables tested:

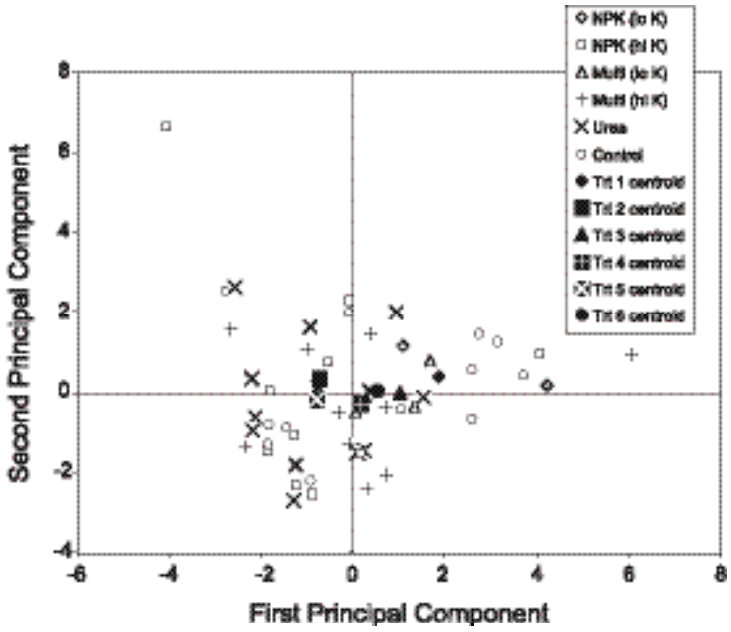


Figure 1. Principal components analysis of foliar chemistry in samples collected from the Balanced Fertilizer Study.

[1] pgng = $a_0 - a_1 \bullet Ca + a_2 \bullet Zn + a_3 \bullet N - a_4 \bullet \ln(Z)$

[2] ln(pgng) = $b_0 + b_1 \bullet K - b_2 \bullet SO_4 - b_3 \bullet \ln(Ca) + b_4 \bullet \ln(Fe) - b_5 \bullet \ln(C) + b_6 \bullet \ln(N)$

[3] norm = $c_0 + c_1 \bullet P - c_2 \bullet Cu - c_3 \bullet Al + c_4 \bullet SO_4 + c_5 \bullet \ln(Fe)$

[4] ln(norm) = $d_0 - d_1 \bullet Cu + d_2 \bullet SO_4 + d_3 \bullet \ln(K) + d_4 \bullet (Fe) - d_5 \bullet (Al)$

Respective R^2 values were 0.56, 0.66, 0.45, and 0.47 for these four models. The negative marginal effect of calcium and the positive effect of nitrogen on unstandardized fungal biomass were consistent with results from unfertilized plots from last year, assuming that higher fungal biomass translates into lower foliage retention. However, neither of these elements displayed any significant relationship to the normalized values. Also, sulfate had a negative relationship with normalized values of fungal mass, but the unstandardized index of fungal mass peaked at an approximate value of 500 ppm of SO_4 in the foliage. In the GIS and PCT control plots analyzed last year, both foliar S and soil S were negatively related to foliage retention.

In summary, the treatments had no effect on fungal biomass in the 2000 foliage of fertilized trees, and had relatively little effect on foliage chemistry 4 to 5 yrs after treatment. Fungal biomass does have a statistically significant relationship to numerous foliar elements, but the patterns are difficult to interpret and do not point to any fertilization treatment that can be expected to ameliorate SNC severity.

Mineral Nutrition and Swiss Needle Cast

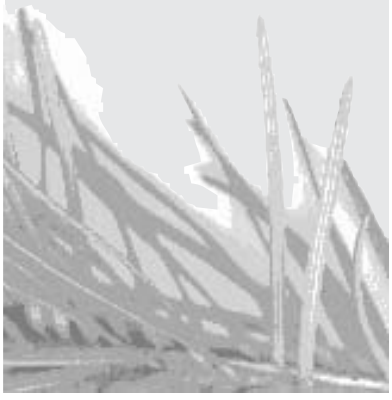
Jeffrey Stone and Paul Reeser

ABSTRACT

The effect of mineral nutrition on needle colonization by the Swiss needle cast pathogen, *Phaeocryptopus gaeumannii*, was investigated in potted Douglas fir seedlings. Seedlings were fertilized with a balanced complete mineral nutrient solution at reduced strength, with one nutrient per treatment added back. Nitrogen, phosphorus, potassium, calcium and sulfur were tested. Supplementation of a basal mineral salts solution with individual nutrients (N, P, K, Ca, S) had no effect on incidence or amount of *P. gaeumannii* colonization. Seedlings provided with supplemental N had reduced needle retention compared to the other treatments.

INTRODUCTION

Mineral nutrition has been suggested as a possible factor influencing the severity of Swiss needle cast disease of Douglas-fir. Mineral nutrient deficiencies or imbalances might interact with disease in several ways. Nutrient deficiencies or imbalances might contribute to disease severity by causing host foliage to be more susceptible to infection or more favorable to fungal colonization. This might occur through alteration of the chemical composition of needles in such a way as to promote growth of the fungal parasite (Rose et al. 2000), or, because of nutrient limitation, preventing the expression of defense responses that might otherwise enable the host to limit infection. Conversely, certain nutrients might interact negatively with disease, reducing infection and colonization by promoting defense responses, or enabling the host to moderate disease symptoms. If mineral nutrition is a significant factor in either the supression or aggravation of the disease, this might lead to new approaches for disease management. To date however, a connection between mineral nutrition and Swiss needle cast disease severity has not been definitively established. This study was undertaken as a preliminary investigation on the relationship between mineral nutrition and Swiss needle cast. Out objectives were to determine whether deficiency or sufficiency of specific macronutrients affect colonization of Douglas-fir foliage by *P. gaeumannii* or symptoms of Swiss needle cast (i.e. chlorosis, premature foliage loss).



MATERIALS AND METHODS

Seedlings

Container grown seedlings (Pelton nursery) from a single seed source were planted in coarse sand in 4 gal pots in Feb, 2000. Seedlings were grouped in three blocks, each block having six treatments with five seedlings per treatment, and maintained under 50% shade at the OSU Botany Field Station until exposure to inoculum at the Salal study site. Treatments were: 1) control 1/2 strength M&S basal salts with no add-back nutrient; 2) add-back N, 1/2 strength M&S basal salts with 3.5 mM additional N; 3) add-back P, 1/2 strength M&S basal salts with 1.94 mM additional P; 4) add-back K, 1/2 strength M&S basal salts with 3.47 mM additional K; 5) add-back Ca, 1/2 strength M&S mineral salts with 1.85 mM add-back Ca; 6) add-back S, 1/2 strength M&S basal salts with 0.21 mM additional S. Seedlings were exposed to inoculum at Salal between May 18 and June 15, 2000, then returned to the OSU Botany Field Station. Seedlings were incubated under a 50% shade cloth from June 15 through Sept 30, and in ambient sunlight thereafter.

Nutrient solutions

Fertilizer treatments were provided as Murashige and Skoog modified basal salts mixture with one half strength NH_4NO_3 and KNO_3 (Sigma M8900). Composition of the nutrient solution was: 5.16 mM NH_4NO_3; 1.50 mM $CaCl_2$; 0.75 mM $MgSO_4$; 4.95 mM KNO_3; 0.63 mM KH_2PO_4; 50µM H_3BO_3; 0.55µM $CoCl_2$-$6H_2O$; 0.50µM $CuSO_4$-$5H_2O$; 15.0µM $ZnSO_4$-7 H_2O; 13.90 mg/L $FeSO_4$-$7H_2O$; 18.63 mg/L Na-EDTA; 2.5µM KI; 1.0 µM Na_2MoO_4-$2H_2O$; 50µM $MnSO_4$-H_2O. The basal nutrient solution was supplemented with an amount of either NH_4NO_3, NaH_2PO_4, KCL, $CaCl_2$-$2H_2O$, or $NaSO_4$ to bring the level of the base nutrient (N, P, K, Ca, or S) to sufficiency. Concentrations of the supplemented nutrients were: 5.0 mM N; 2.0 mM P; 4.0 mM K; 2.0 mM Ca; 0.3 mM S. The complete composition of fertilizer solutions for each treatment is shown in Table 1. The concentration of nutrients supplemented was based on Walker and Gessel (1991). All seedlings received 100 mL of 1/2 strength M&S basal salts and 100 mL of the add-back nutrient solutions per pot at weekly intervals from May through August, 2000, and monthly thereafter until sampling in April 2001. Control seedlings received only 100 mL water instead of the add-back.

Disease assessment

Foliage from the exposed seedlings was collected in April 2001. Two shoots bearing needles of the 2000 needle complement were clipped, the needles stripped from the shoots, and a sample of 50 drawn randomly. Needles were affixed to 3 x 5″ index cards with double sided adhesive tape for examination. Infection incidence was determined as the proportion of needles bearing pseudothcia of *P. gaeumannii*. Pseudothecial density

Table 1. Composition of fertilizer solutions, based on Murashige and Skoog (1962). Nutrient concentrations that are different from other treatments are highlighted.

	Add-back nutrient					
	Control	Nitrogen	Phosphorus	Potassium	Calcium	Sulfur
NH_4NO_3	5.16 mM	**22.6 mM**	5.16 mM	5.16 mM	5.16 mM	5.16 mM
$CaCl_2$	1.50 mM	1.50 mM	1.50 mM	1.50 mM	**20.0 mM**	1.50 mM
$MgSO_4$	0.75 mM	0.75 mM	0.75 mM	0.75 mM	0.75 mM	0.75 mM
KNO_3	4.95 mM	4.95 mM	4.95 mM	4.95 mM	4.95 mM	4.95 mM
KH_2PO_4	0.63 mM	0.63 mM	0.63 mM	0.63 mM	0.63 mM	0.63 mM
H_3BO_3	50.0 µM	50.0 µM	50.0 µM	50.0 µM	50.0 µM	50.0 µM
$CoCl_2$-$6H_2O$	0.55 µM	0.55 µM	0.55 µM	0.55 µM	0.55 µM	0.55 µM
$CuSO_4$-$5H_2O$	0.50 µM	0.50 µM	0.50 µM	0.50 µM	0.50 µM	0.50 µM
$ZnSO_4$-7 H_2O	15.0 µM	15.0 µM	15.0 µM	15.0 µM	15.0 µM	15.0 µM
Na_2MoO_4-$2H_2O$	1.0 µM	1.0 µM	1.0 µM	1.0 µM	1.0 µM	1.0 µM
$FeSO_4$-$7H_2O$	13.9 mg/L	13.9 mg/L	13.9 mg/L	13.9 mg/L	13.9 mg/L	13.9 mg/L
Na-EDTA	18.6 mg/L	18.6 mg/L	18.6 mg/L	18.6 mg/L	18.6 mg/L	18.6 mg/L
KI	2.5 µM	2.5 µM	2.5 µM	2.5 µM	2.5 µM	2.5 µM
$MnSO_4$-H_2O	50.0 µM	50.0 µM	50.0 µM	50.0 µM	50.0 µM	50.0 µM
Na_2SO_4						**2.10 mM**
NaH_2PO_4-H_2O			**19.4 mM**			
KCl				**34.7 mM**		

was determined from a subset of 10 needles bearing pseudothecia. The number of pseudothecia in a row of stomata from the petiole, middle, and apical portions of the needle was counted under a dissecting microscope fitted with a counting grid. The total pseudothecia in the three segments were averaged for each needle, and the average of ten needles calculated for each tree. The amount of *P. gaeumannii* in samples of ten needles per tree was also determined by the quantitative PCR procedure (Winton et al in press). Needle retention was estimated in Sept 2001 by means of a visual rating scale where 9 = 91-100% of needles retained, 0 = 0-10% of needles retained.

RESULTS

Composition of the nutrient medium had no effect on incidence or severity of infection of seedlings as assessed by presence and abundance of pseudothecia (Figs.1, 2). Differences in fungal biomass in foliage were not found by quantitative PCR analysis (Fig. 3). One treatment, the add-back N treatment, had more foliage loss by Sept 2001 than the other treatments (Fig 4).

DISCUSSION

This experiment was designed as a preliminary test of whether an effect of an imposed nutritional imbalance could affect infection and colonization of Douglas-fir foliage by *P. gaeumannii*. The results of this experiment do not demonstrate promotion or inhibition of *P. gaeumannii* infection or colonization of Douglas-fir foliage by macronutrients under the conditions employed here. However, since these results were obtained over only a single year, they can not be considered conclusive. It is possible that a longer period of nutritional preconditioning is necessary to establish a nutritional imbalance sufficient to manifest differences in fungal colonization. These results

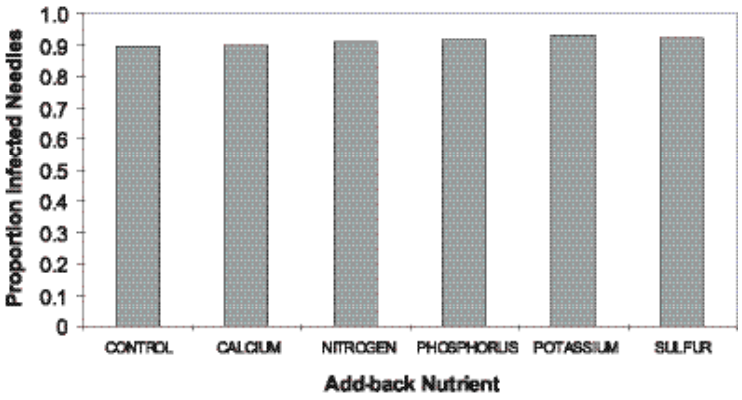


Figure 1. Incidence of infection in seedlings treated with a reduced strenght nutrient solution with one nutrient supplemented to sufficiency (add-back

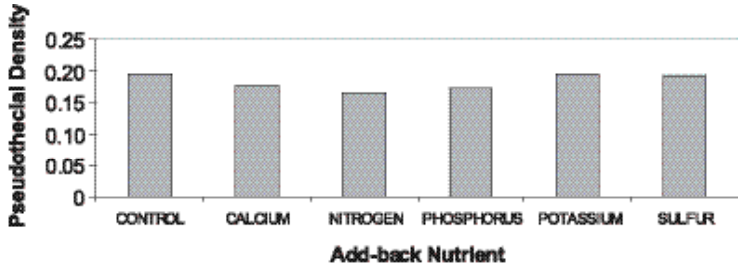


Figure 2. Infection severity (proportion of stomata occupied by pseudothecia) in seedlings treated with a reduced strenght nutrient solution with one nutrient supplemented to sufficiency (add-back nutrient).

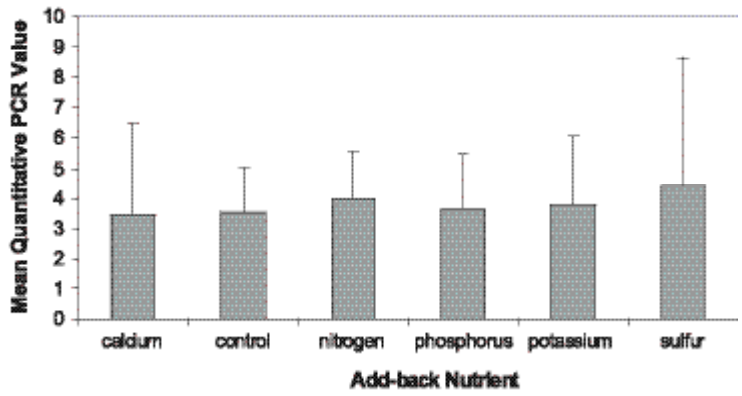


Figure 3. Treatment means and standard deviations for quantitative PCR analysis of P. gaeumannii *in foliage.*

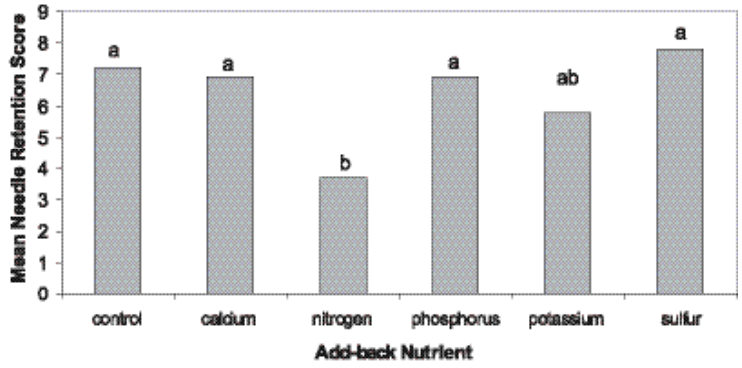


Figure 4. Needle retention in seedlings treated with a reduced strenght nutrient solution with one nutrient supplemented to sufficiency (add-back nutrient). Needle retention was estimated by a rating scale of 1 – 9, where 9 = 90-100% of needles retained.

only suggest that such effects can not be detected in treatments applied during single growing season.

The initial applications of fertilizer treatments coincided with bud break and continued through the inoculation period. The nutritional conditions experienced by the seedlings during the preceeding growing season in the nursery may have had a greater influence on the availability of nutrients to the needles early in their development than the fertilizer treatments. The main effects of fertilizer treatments, if any, on the needles therefore most likely occurred during the post inoculation period during when active fungal colonization took place. If differences in nutritional composition of needles due to treatments affected fungal colonization, this should have been revealed as differences in pseudothecial density or fungal biomass as determined by quantitative PCR analysis of needles.

The only effect due to treatments seen in this study was greater needle loss after one growing season with N supplementation. Since the amount of fungal colonization in this treatment was very similar to the other treatments, the reason for greater foliage loss is not known. Typically there is very little foliage lost after the first growing season, even where SNC is severe, so the level of defoliation seen in the add-back N treatment is unusual.

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Effects of Fertilization and Vegetation Control on Swiss Needle Cast Infection and Growth of Coastal Douglas-fir Saplings

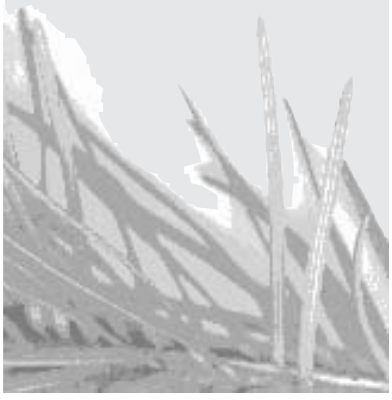
Gabriel A. Crane, Robin Rose, Scott Ketchum, and Diane Haase
Cooperators: Jeff Stone and Wendy Sutton

Abstract

This study was initiated in May 1999 to determine if silvicultural treatments (fertilization and vegetation control) could enhance the growth of Douglas-fir saplings and/or alleviate SNC infection. Current results indicate that the removal of competing vegetation on all study sites has a significant positive effect on mean DBH growth (16% - 19%) when compared to the non-removal treatments. However, no significant differences in height growth based on vegetation treatments have been documented. Fertilizer treatments have had no significant impact on height or DBH growth. There was a significant interaction between fertilizer and vegetation control at the Bushy Peterson site with regard to SNC infection (p-value=0.0077). No significant differences with regard to infection levels were found at the other two respective sites. At all sites there were no consistent foliar nutrient concentration responses to fertilization other than for boron.

Introduction

The potential to increase vigor and growth within Douglas-fir sapling plantations that are affected with Swiss needle cast (SNC) through silvicultural manipulation is currently unknown. Vegetation control and fertilization and/or their interactions in altering SNC infection and increasing biomass growth are of considerable theoretical and practical interest. Vegetation control is a basic concern in forest stand management (Opio et al. 2000). Both intraspecific and interspecific competition can decrease resource availability, thereby reducing tree growth in comparison with that of trees without competition (Cole and Newton 1987). It has been demonstrated that changes in nutrient element dynamics due to fertilization can have a significant impact on biomass production and overall growth (Mitchell et al. 1995).



Materials and Methods

Field Sites

Three field trials were conducted at three different sites installed across an east/west transect of the Oregon Coast Range. Installation of study replicates across this gradient was chosen because the greatest level of

growth loss associated with SNC has been found in the coastal regions and tends to decrease eastward toward the Willamette Valley. The main reason for this is that eastward from the coast, drier conditions are approached, which are not conducive to SNC.

The site nearest the coast is located between Toledo and Siletz (South Drake). This particular site is on The Timber Company's ownership. The second site is midway between the coast and the Willamette Valley near Eddyville (Bushy Peterson). This site is located on Starker Forest lands. The third site is on the western valley fringe close to Summit (Charlie Olson). This site is on Starker Forest lands as well. Each site had existing 6-year-old Douglas-fir plantations when the experiment was implemented.

Experimental Design and Treatments

This experiment consists of three separate study sites (Charlie Olson, Bushy Peterson, and South Drake). At each site the experiment is a randomized complete block design with five replications (blocks) of each treatment plot. An exception to this is the Bushy Peterson site where there are only four replications due to limited space. The six treatments consist of a 2 X 3 factorial design with two levels of weed control and three levels of fertilization. Each treatment plot is 70 X 70' and encompasses 25-35 operationally planted trees of which the center most 15 trees are identified for evaluation in the experiment.

There were two vegetation control treatments:

1) No control
2) Control of all competition for three consecutive years

All treatment plots by block were assigned at random. On September 7-9, 1999, all woody vegetation on the South Drake and Bushy Peterson sites was manually cut to ground level. The Charlie Olson site did not have a significant amount of woody vegetation and therefore did not necessitate manual control of woody vegetation. In addition, vegetation free plots received an herbicide application broadcast with a backpack sprayer. Fall application of Sulfo-meturon (Oust) at 3 oz/a and Glyphosphate (Accord) at 1.5 qt/a was used to eliminate all woody and herbaceous competitors. Additional spring and fall applications of herbicide were used to maintain weed-free conditions.

The three fertilizer treatments were:

1) Unfertilized control
2) 400 grams of 9-17-17 per tree / application
3) 400 grams of 18-17-17 per tree / application

The six-month controlled-time release fertilizer formulations with minor elements included were from Simplot Company and were identical with the exception of nitrogen content. Applications were made initially on September 8-10, 1999 to each of the 15 trees in the plots designated for fertilization. Fertilizer blends were surface applied around the base of the tree. Fertilizer was tossed by hand into the middle of the tree at approximately two feet off of the ground and allowed to scatter as it fell through the branches. Fertilizer applications were made again in April and October 2000, as well as April 2001.

Measurements and Sampling

Initial diameter at breast height (DBH) and height were measured in May 1999. Measurements were also recorded in October 1999 and 2000. Height measurements were taken using calibrated Crane™ 11m telescoping fiberglass rods. DBH measurements were taken with Spencer™ diameter tapes. Seasonal growth was calculated by subtracting the previous years growth from the current years growth. Height to diameter ratio (HDR) is an individual tree based index, and is calculated by dividing the height of the measure tree by the DBH of the tree.

An initial SNC assessment was made in mid-July 1999 on a branch at breast height. This same branch was used for continuing assessments of SNC infection. Assessments are made on a yearly basis during May of each year from the same branch where the previous assessment was taken. The assessment consisted of ocularly estimating the percentage of needle retention for each cohort of needles on the main stem of the branch and also on a lateral marked for future assessments. When monitoring the disease on an individual tree level, needle retention has correlated well with disease severity (Filip et al. 2000). By tagging and using the same branches for monitoring purposes every year, it was possible to follow the progression or decline of the disease and its' severity as the case may be.

In February 2001, current-year foliage was sampled at all sites on an individual tree basis. Five sub-samples were taken from the top one-third of the crown from each individual tree. The sub-samples were pooled and analyzed on an individual tree basis. Using the Polymerase Chain Reaction (PCR) procedure, the relative amount of *Phaeocryptopus gaeumannii* fungus within needles was identified. PCR results in a ratio of picograms of *Phaeocryptopus* DNA to nanograms of Douglas-fir DNA. PCR is being used because it has the advantage of speed, technical simplicity, low detection limits, and specificity over other procedures (SNCC Annual Report 1999). This procedure has the ability to determine the exact level of fungus within an individual needle.

Initial foliar and soil nutrient analyses (data not shown) were performed on samples from each site in late October 1998. One and two-year-old foliage was collected from mid-canopy on 10 trees at each site, and a composite sample from each site were sent for analyses.

During December 2000, three sub-samples of the current year's foliage were collected from six trees from the top one-third of the crown from each treatment replication for foliar nutrient analyses. All foliage samples were dried for 72 hours at 70° C. Needles were removed from the pooled sample and the weight of 100 needles determined for each treatment replication. Dry weight was measured on a sample of 100-needles/pooled sample for use in calculating nutrient contents and vector analysis (Haase and Rose 1995). Foliage was then ground in a Wiley mill grinder (40 mesh screen). Nutrient concentrations (USAg Analytical Services, Inc., Pasco, WA) were determined from foliage samples using standard laboratory procedures. Relative nutrient concentration, content, and dry weight were calculated (relative to the control treatments) and vector diagrams constructed to facilitate a thorough examination of nutrient responses to the respective treatments.

A sample of 100 needles from each treatment replicate was weighed to obtain a unit dry weight for determination of nutrient contents and facilitation of vector analysis (Haase and Rose 1995). The incorporation of a growth parameter with nutrient concentrations and contents into a vector diagram enables rapid evaluation of treatment effects (Timmer and Stone 1978, Haase and Rose 1995). A reference point is chosen (the control treatment in this study) and set equal to 100. Subsequent data points are thus normalized to the reference point. Interpretations of the significance of the nutrient shift (dilution, sufficiency, deficiency, luxury consumption) can be made based on the relative changes in nutrient content, concentration, and unit dry weight (i.e. the direction and magnitude of the vector). The vector diagrams in this study compare nutrient shifts relative to one reference point (the control treatment). Vector diagrams were constructed for the 2000 foliar nutrient samples.

Results and Discussion

Morphology

On all sites, complete vegetation removal (spray) had a significant positive effect on mean 2000 DBH growth (p-values<.0001), when compared with the non-removal treatment (check) (Figure1). The Charlie Olson site had an increased annual DBH growth of 16%, while both the Bushy Peterson and South Drake sites had annual increases of 19%. Height growth was not significantly different between the two respective vegetation treatments. In addition, no fertilizer treatment had a significant effect on either mean DBH or height growth.

Height to diameter ratios were significantly different between the complete vegetation removal treatments and the non-removal treatments at each respective site after two full growing seasons (Figure 2). At the Charlie Olson site the ratio was in-

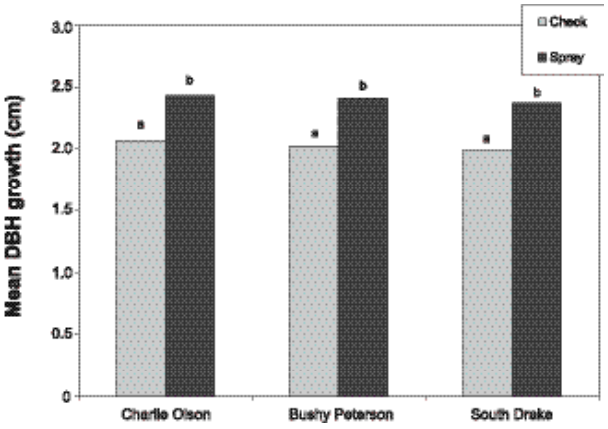


Figure 1. Mean 2000 DBH growth (cm). Bars associated with a different letter from the same site are significantly different at the α≤0.05 level. All sites were analyzed independently of each other.

creased by 7.5% between treatments (p-value=.0293). The Bushy Peterson site exhibited an increased ratio that differed by 5.7% (p-value=0.0004). The South Drake site was similar to the other two sites with an increased ratio, and a 5.4% difference between the two respective treatments (0.0056). The fertilizer treatments had no significant impact on height to diameter ratios.

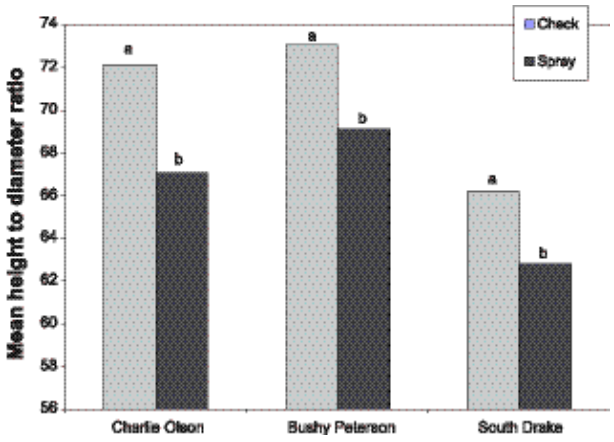


Figure 2. Mean 2000 height to diameter ratios. Bars associated with a different letter from the same site are significantly different at the α≤0.05 level. All sites were analyzed independently of each other.

Sapling Needle Weights

At the three respective study sites, needle weights were greatest in plots where complete vegetation removal was implemented. There were significant treatment differences between the complete vegetation removal treatments and the non-removal treatments with regard to mean weight per 100 oven-dried needles for the 2000 cohort needles on the Charlie Olson (p-value=0.0243) and Bushy Peterson (p-value=0.0038) sites (Table 1). The South Drake site did not demonstrate significant differences among any treatments (p-value>0.05). However, there were distinct trends between the complete vegetation removal treatment and the control treatment at South Drake.

At the Charlie Olson site a 7.3% increase in needle weight was recognized with complete vegetation removal. The greatest difference in needle weights between vegetation treatments occurred at the Bushy Peterson site, where a 12% increase was observed. The South Drake site was similar to the Charlie Olson site with a respective 5.2% increase. Fertilization played no significant role in needle weight results.

SNC Infection (PCR Analyses)

There was a significant interaction between fertilizer and vegetation control at the Bushy Peterson site with regard to SNC infection (p-value=0.0077). A post hoc comparison of treatments at Bushy Peterson based on a restrictive means comparison (Tukey-Kramer) resulted in no significant differences between treatments (p-values>0.05). No significant treatment differences were found at the other two respective sites (Figures 3a,b, c). However, it is interesting to note that based on PCR analyses, the Bushy Peterson site was more heavily infected on average than the South Drake site. This is especially interesting for two distinct reasons. First, the Bushy Peterson site is located approximately 25 miles inland from the coast, whereas the South Drake site is within five miles of the coast with each nearly lying on the same latitudinal line. It is commonly thought that the highest SNC infection levels are within 15 miles of the coast. Secondly, despite having an infection level that is 34% less than the Bushy Peterson site, the South Drake site has an average lower needle retention (Figure 4). The increased infection at the Bushy Peterson site could potentially be why an interaction effect was revealed.

Table 1. 2000 mean sapling needle weights (grams). Means with different letters within the same site are significantly different at the α≤0.05 level. Standard error of the mean is in parentheses. All sites were analyzed independently of each other.

Site	Treatment	2000 Cohort
Charlie Olson	Check	0.55 (.01) a
	Spray	0.59 (.01) b
		p-value=0.0243
Bushy Peterson	Check	0.58 (.01) a
	Spray	0.65 (.01) b
		p-value=0.0038
South Drake	Check	0.57 (.01) a
	Spray	0.60 (.01) a
		p-value=0.1051

Foliar Nutrients

At all sites there was no consistent foliar nutrient concentration response to fertilization other than for boron. Due to this general non-response to the fertilization treatments, the six-month time released blend was changed to a soluble form in the spring of 2001. In addition, the rate per individual tree was increased by 45% to 580 grams/tree. Current year foliage will be sampled again during December of 2001.

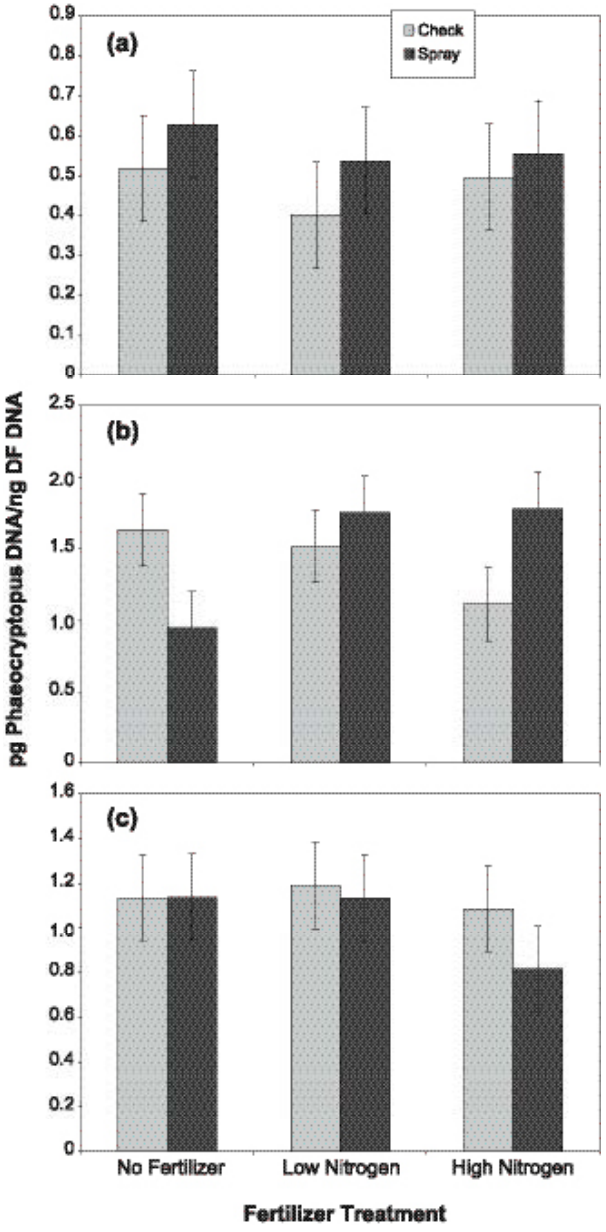


Figure 3. Polymerase Chain Reaction (PCR) results. (a) Charlie Olson; (b) Bushy Peterson; and (c) South Drake. All sites sampled on February 5, 2000. Error bars are the standard error of the mean. All sites were analyzed independently of each other.

At the Charlie Olson site there were significant differences in nitrogen between the two vegetation treatments (p-value=0.0016) (Figure 5). An 8.4% increase in foliar nitrogen was recognized in the complete removal treatments when compared to the non-removal treatments. The other two sites did not exhibit significant differences in foliar nitrogen (p-values >0.05), but they did demonstrate a similar trend to the Charlie Olson site in that their nitrogen levels increased by 4.3% and 4.5%, respectively.

Vector analysis

Vector diagrams constructed on the 2000 foliar age class showed a similar distinct pattern with nitrogen over all three study sites (data not shown). At the Charlie Olson and Bushy Peterson sites the relative nitrogen contents increased with vegetation removal treatments by approximately 25 %. At the South Drake site there was a more moderate increase in nitrogen content of 15 %.

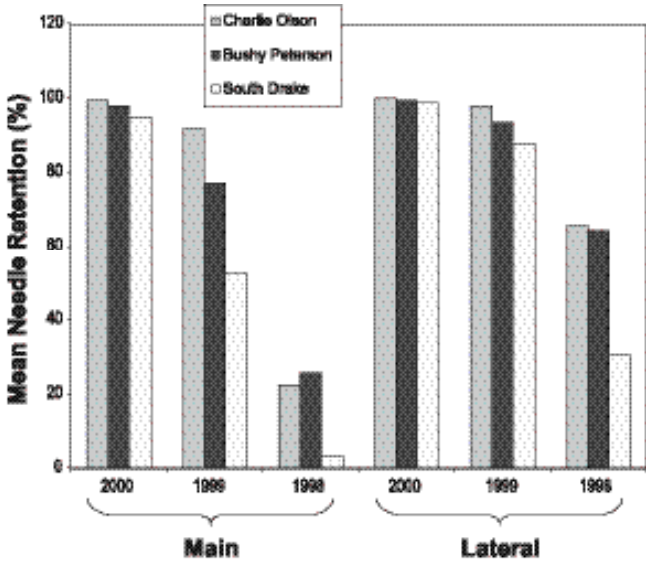


Figure 4. Mean needle retention by site; May 2001.

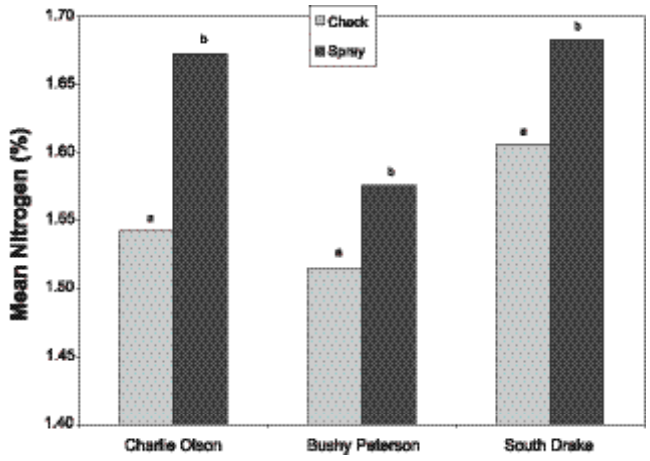


Figure 5. 2000 mean foliar nitrogen (%). Bars associated with a different letter from the same site are significantly different at the $\alpha \leq 0.05$ level. All sites were analyzed independently of each other.

Relative nitrogen concentration was increased slightly by vegetation removal treatments at the Charlie Olson site (10 %). No meaningful concentration increases were observed at the Bushy Peterson or South Drake sites. Fertilization treatments showed no increases in either relative concentration or content. Relative needle dry weights increased over all treatments at each respective site when compared to the control treatment. The vegetation removal treatments generally had the greatest relative weights.

Of the remaining nutrients tested; boron, zinc, and copper showed similar vector patterns over all three sites (data not shown). In general, the relative content of these nutrients was increased 20-25 % when compared to the control treatment. Complete vegetation removal treatments generally had slightly increased content levels when compared to the fertilizer with no vegetation removal. All other nutrients that had vector diagrams constructed showed no distinct patterns among treatments (phosphorus, potassium, calcium, magnesium, and sulfur) (data not shown).

Xylem Water Potential

On August 8-10, 2000, diurnal xylem water potentials were measured on each site. No trends between different treatments and xylem water content were found. It was observed that the most heavily infected site, South Drake, had the greatest amount of moisture stress during pre-dawn measurements when compared to the other two sites. However, this same site showed the least amount of moisture stress during the highest stress period (1-3 pm). Increased soil moisture may in part be responsible for the lower overall xylem water potentials at this site.

Soil Moisture Content

Soil samples from all sites were taken August 8-10, 2000 in order to determine soil moisture contents. Total soil moisture content (%) increased on an east to west gradient (38%, 25%, and 23%, respectively). There were no trends found between individual treatments and soil moisture (%).

Vegetation Survey

A vegetation survey was conducted in July 2000 in order to ensure that the complete vegetation removal treatments had good efficacy over all three sites. A stratified rather than random vegetation survey was conducted because many times vegetative species clump together. It is therefore more accurate to spread plots in order to gain a better understanding of the overall vegetative population.

Vegetation cover over all three sites was reduced by approximately 85% in the complete vegetation removal plots. In addition, vegetation height was reduced by approximately 75%. Virtually all vegetation that remained consisted of herbaceous species. Shrubs and woody competitors were successfully eliminated from all complete vegetation removal plots.

Conclusion

Current research dictates that vegetation removal in sapling Douglas-fir stands is beneficial. A positive growth response (increased DBH growth) and no increased SNC infection within treatments make vegetation control a potentially useful management tool in young Douglas-fir plantations. However, in operational settings it may be difficult to obtain the same levels of vegetation control as exhibited in this study depending on the type of vegetation that is present. With more time it will be possible to determine the lasting effects that vegetation control has with regard to current documented growth responses. Research continues to be ongoing with fertilizers, specifically with a focus on potential growth responses and nutrient effects on fungal colonization.

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Effect of Elemental Sulfur on Swiss Needle Cast Infection and Growth of Coastal Douglas-fir Saplings

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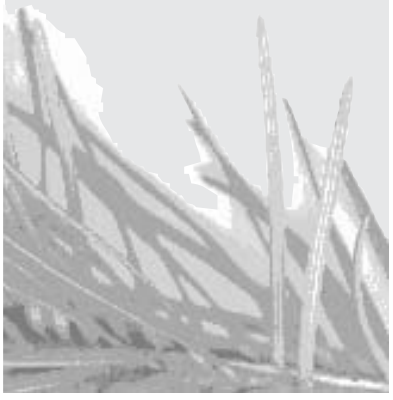
Cooperators: Jeff Stone and Wendy Sutton

Abstract

The objective of this experiment was to determine how elemental sulfur (thiolux) and Bravo fungicide affect Swiss needle cast (SNC) infection and subsequent growth in Douglas-fir saplings. Concurrently, foliar and soil nutrient change and needle retention were of interest. Thiolux was applied as a foliar application with and without TacTic sticker on individual trees in an existing 6-year-old *Pseudotsuga menziessii* plantation in the Oregon Coast Range five miles north of Toledo, OR. Additionally, a thiolux ground and foliar Bravo fungicide application were applied. Bravo fungicide applied at a rate of 3.75 pts/100 gallons of water sprayed on the foliage for 14 seconds resulted in a significant reduction in infection when compared to all other treatments. The thiolux with-sticker was also significantly effective at lowering *P. gaeumannii* levels when compared to the control treatment but less effective than the Bravo treatment. All other treatments were not significant in lowering *P. gaeumannii* levels when compared to the control treatment. Trees that received the Bravo fungicide treatment had significantly greater foliar nitrogen levels when compared to the control treatment. Foliar treatment applications of both thiolux with and without sticker led to significantly increased levels of foliar sulfur. Height and diameter at breast height (DBH) were not significantly affected by any treatment. Needle retention was not significantly different on the treated needle main or lateral branches 19 months after the initial treatment.



Introduction

Over the past 10 years there has been an increasing incidence as well as damage due to SNC in the Oregon Coast Range. There is an immediate need to find a remedy to increase the overall growth and vigor of these infected Douglas-fir plantations. In 1997, an anecdotal experiment using elemental sulfur (thiolux) suspended in water and sprayed on the trees in an unknown amount increased sapling basal area growth by 18% after two years. Those results were encouraging enough to warrant further investigation to better understand the potential for elemental sulfur

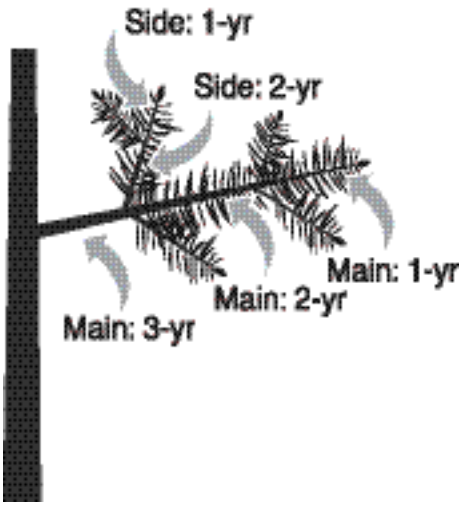


Figure 1. Needle cohort retention assessment diagram.

applications to lessen SNC impacts on Douglas-fir saplings. Additionally, this study is focused on infection severity measured through both needle retention and quantitative real-time PCR (polymerase chain reaction) assay developed specifically for quantification of *P. gaeumannii*. Foliar nutrient status, specifically nitrogen and sulfur were monitored to determine possible needle absorption of sulfur from foliar applications or sulfur translocation via the xylem from ground treatments.

Materials and Methods

Study Site

The sulfur study site was installed near Toledo, Oregon in an existing six-year-old Douglas-fir plantation in May 1999. The plantation had a stocking density of 310 trees/acre. It is located in T. 10S. R. 10W. Sec. 19. The soils are Tolovana of the Reedsport Association. The site index (50) is 136. The study was implemented on industrial land owned by The Timber Company.

Experimental design and treatments

This experiment utilizes a complete randomized design with 10 replications (trees) for each of the five treatments. Treatments were assigned randomly to trees utilizing the entire site. Selected trees were at least 6m apart to avoid treatment drift from nearby applications. Trees with forked stems or which originated from natural regeneration were not used for measurement trees.

There are five sulfur treatments in the study:

1) Untreated control
2) Bravo™ fungicide @ 3.75 pts/100 gallons sprayed on the foliage
3) Sulfur (Thiolux™) diluted with water (25 lb per 100 gallons) sprayed on the foliage
4) Sulfur (Thiolux™) diluted with water (25 lb per 100 gallons) with TacTic™ sticker (8 oz per 100 gallons) sprayed on the foliage
5) Sulfur (Thiolux™) diluted with water (25 lb per 100 gallons) sprayed on the ground under each tree within the drip line

Treatments were applied on June 8, June 25, and July 10, 1999 using a truck tank sprayer at 38 psi. Each tree was sprayed evenly top to bottom for 14 seconds resulting in an application rate of 2 oz Thiolux per tree. This resulted in a "wetting" of the needle surface area. Treatments were applied at two week intervals that corresponded with shoot elongation. This is because the peak period for needle infection occurs during the time of elongation when there is new needle growth and plentiful moisture creating ideal infection conditions.

Measurements and Sampling

Initial diameter at breast height (DBH) and height were measured in May 1999. Measurements were also recorded in October 1999 and 2000. Seasonal growth was calculated by subtracting the previous years growth from the current years growth. Height to diameter ratio (HDR) is an individual tree based index, and was calculated by dividing the height of the measure tree by the DBH of the tree.

An initial SNC assessment was made in mid-July 1999 on a branch at breast height. This same branch was used for continuing assessments of SNC infection. Assessments are made on a yearly basis during May of each year from the same branch where the previous assessment was taken. The assessment consisted of ocularly estimating the percentage of needle retention for each cohort of needles on the main stem of the branch and also on a lateral marked for future assessments (Figure 1). When monitoring the disease on an individual tree level, needle retention has correlated well with disease severity (Filip et al. 2000). By tagging and using the same branches for monitoring purposes every year, it was possible to follow the progression or decline of the disease and its severity as the case may be.

In April 2000, current-year foliage was sampled on all 50 trees. Five samples were taken from the top one-third of the crown of each individual tree. The samples were pooled by tree and the pooled sample used for analysis. Using the Polymerase

Chain Reaction (PCR) procedure, the relative amount of *Phaeocryptopus gaeumannii* fungus within needles was identified. PCR results in a ratio of picograms of *Phaeocryptopus* DNA to nanograms of Douglas-fir DNA. PCR was used because it has the advantage of speed, technical simplicity, low detection limits, and specificity over other procedures (SNCC Annual Report 1999). This procedure has the ability of determining the exact level of fungus within an individual needle.

In February 2001, both current-year (2000 cohort) and second-year (1999 cohort) foliage were sampled on all 50 trees in order to evaluate the effectiveness of treatments after one full growing season with respect to infection levels. PCR analyses were implemented on these samples.

Foliar and soil nutrient samples were collected in December of 1999. Current year foliage was collected from mid-canopy on four random trees from each treatment. Three sub-samples from each tree were collected and pooled for analyses. Soil was collected from the base of the same four trees at three different locations within the drip line. Soil nutrient concentrations (Oregon State University Central Analytical Laboratory) were determined from soil samples using standard laboratory procedures. The soil from the three locations per tree was mixed to obtain one characteristic sample from each tree. Soil was collected from the first six inches of the A-horizon.

During December 2000, three sub-samples of the current year's foliage were collected from all treatment trees at mid-canopy. The three sub-samples collected were pooled by individual tree for analyses.

All foliage samples were dried for 72 hours at 70° C. Needles were removed from the pooled sample and the weight of 100 needles determined for each individual tree. Dry weight was measured on a sample of 100 needles/pooled sample for use in calculating nutrient contents and vector analysis (Haase and Rose 1995). Foliage was then ground in a Wiley mill grinder (40 mesh screen). Nutrient concentrations (USAg Analytical Services, Inc., Pasco, WA) were determined from foliage samples using standard laboratory procedures.

Results

General Results

Sapling morphological development (height and DBH) was not significantly affected two-years after treatment. Additionally, height to diameter ratios were not significantly different among treatments.

Six months after the treatments were applied there was no significant difference in soil nutrients among treatments. Saplings that received the Bravo fungicide treatment demonstrated the largest increase in foliar nitrogen levels after six months when compared to the control.

Bravo was the most effective treatment in reducing the colonization of *P. gaeumannii* in treated foliage (1999 cohort). The 2000 needle cohort showed no significant differences among treatments. There were no treatment effects on mean weight per 100 oven dried needles for the 1999 cohort needles sampled during December of 1999 or the 2000 cohort needles sampled in December 2001. There were no significant differences in mean needle retention between treatments for both the May 2000 and May 2001 needle assessments.

Morphology

There were no significant differences among treatments in DBH or height growth after the first growing season (1999) (Table 1). These results were expected, as the respective treatments did not yet have time to integrate treatment effects into significant growth impacts. Two full growing seasons (Fall 2000) after the treatments were implemented there were no significant differences

Table 1. Mean 1999 and 2000 height and DBH growth. Treatments within columns associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

	1999		2000	
Treatment	DBH Growth (cm)	Height Growth (cm)	DBH Growth (cm)	Height Growth (cm)
Control	1.69 (0.09) a	98.1 (8.7) a	2.22 (0.15) a	112 (8.6) a
Bravo	1.80 (0.09) a	98.4 (8.7) a	2.26 (0.15) a	107.5 (8.6) a
Sulfur w/ sticker	1.88 (0.09) a	103.5 (8.7) a	2.34 (0.15) a	103.5 (8.6) a
Sulfur w/o sticker	1.91 (0.09) a	100.8 (8.7) a	2.2 (0.15) a	101.5 (8.6) a
Sulfur ground	1.64 (0.09) a	102.1 (8.7) a	1.98 (0.15) a	92 (8.6) a
P > F	0.1968	0.9904	0.5264	0.5624

recognized among treatments in DBH or height growth (Table 1). Additionally, there were no significant differences in height to diameter ratios between treatments after two growing seasons.

Soil Nutrients

There were no significant differences (p-values≥0.1) among treatments for any of the soil nutrients tested in December 1999 (Table 2). However, the thiolux ground application nearly doubled the control treatment when looking specifically at SO_4 ppm.

Foliar Nutrients

Trees that received the Bravo fungicide treatment had significantly greater nitrogen levels than the control treatment (p-value=.0037) six months after treatment (Table 3). The thiolux foliage application also had moderately significant increases in foliar nitrogen when compared to the control treatment (p-value=.01). All other treatments were not significantly different from the control.

The thiolux with-sticker and thiolux with-no sticker application differed significantly from the control treatment when comparing sulfur nutrient levels six months after treatment (p-values=.0003 and .0043, respectively). All other nutrients tested did not differ significantly from the control treatment.

The 2000 needle cohort nitrogen levels were significantly different in the sulfur (p-value=.0061) and sulfur ground (p-value=.0332) treatments when compared to the control treatment 19 months after treatment applications (Table 4). The Bravo treatment, which had shown a significant response after six months was not significantly different from the control 19 months after treatment.

Sulfur levels were increased significantly from the control treatment for all treatments except Bravo, which was only marginally significantly increased (p-value=.0572). Additionally, the Bravo treatment had significant increases in boron when compared to the control treatment (p-value=.0007) (Table 4). All other nutrients showed no significant increases among treatments.

Table 2. 1999 mean soil nutrients. Treatments associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatment	N %	P ppm	K ppm	SO_4 ppm	Ca meq 100g	C %	pH
Control	0.66 (0.06) a	6.75 (0.32) a	405.8 (32.1) a	17.28 (3.5) a	1.3 (0.24) a	11.93 (.92) a	4.6 (0.07) a
Bravo	0.54 (0.06) a	5.75 (0.32) a	364.5 (32.1) a	22.28 (3.5) a	0.8 (0.24) a	9.7 (0.92) a	4.73 (0.07) a
Sulfur w/ sticker	0.68 (0.06) a	5.75 (0.32) a	371.3 (32.1) a	27.25 (3.5) a	1.6 (0.24) a	12.8 (.92) a	4.58 (0.07) a
Sulfur w/o sticker	0.63 (0.06) a	5.67 (0.36) a	376.7 (35.9) a	20.47 (3.9) a	1.1 (0.27) a	11.2 (1.03) a	4.57 (0.07) a
Sulfur ground	0.59 (0.06) a	5.5 (0.32) a	380.3 (32.1) a	31.4 (3.5) a	0.98 (0.24) a	11.48 (.92) a	4.58 (0.07) a
P > F	0.645	0.295	0.964	0.265	0.527	0.496	0.6768

Table 3. 1999 Foliar Nutrients. Treatments associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatments	N %	P %	K %	S %
Control	1.52 (0.06) a	0.15 (0.01) a	0.73 (0.06) a	0.07 (0.01) a
Bravo	1.82 (0.06) c	0.17 (0.01) a	0.68 (0.06) a	0.08 (0.01) a
Sulfur w/ sticker	1.60 (0.06) ab	0.17 (0.01) a	0.70 (0.06) a	0.13 (0.01) b
Sulfur w/o sticker	1.78 (0.06) bc	0.19 (0.01) a	0.73 (0.06) a	0.11 (0.01) b
Sulfur ground	1.68 (0.06) abc	0.17 (0.01) a	0.7 (0.06) a	0.09 (0.01) a
P > F	0.0205	0.0942	0.9692	0.0015
	Ca %	**Mg %**	**Na %**	**B ppm**
Control	0.25 (0.05) a	0.09 (0.01) a	0.07 (0.05) a	14.03 (1.5) a
Bravo	0.39 (0.05) a	0.12 (0.01) a	0.05 (0.05) a	17.55 (1.5) a
Sulfur w/ sticker	0.36 (0.05) a	0.10 (0.01) a	0.05 (0.05) a	17.03 (1.5) a
Sulfur w/o sticker	0.32 (0.05) a	0.09 (0.01) a	0.07 (0.05) a	18.08 (1.5) a
Sulfur ground	0.41 (0.05) a	0.12 (0.01) a	0.22 (0.05) a	14.98 (1.5) a
P > F	0.2144	0.2442	0.0841	0.3122
	Zn ppm	**Mn ppm**	**Fe ppm**	**Cu ppm**
Control	18.75 (3.3) a	317.5 (82) a	106.25 (22.49) a	5.25 (0.93) a
Bravo	25.25 (3.3) a	391.8 (82) a	104.00 (22.49) a	6.50 (0.93) a
Sulfur w/ sticker	23.50 (3.3) a	342.5 (82) a	136.25 (22.49) a	6.50 (0.93) a
Sulfur w/o sticker	21.00 (3.3) a	462.5 (82) a	78.50 (22.49) a	7.25 (.93) a
Sulfur ground	28.75 (3.3) a	523.75 (82) a	92.50 (22.49) a	6.75 (0.93) a
P > F	0.2941	0.40	0.4870	0.6523

Table 4. 2000 foliar nutrients. Treatments associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatments	N %	P %	K %	S %
Control	1.552 (0.05 a	0.155 (0.006)a	0.706 (0.03)a	0.102 (0.003)a
Bravo	1.599 (0.05)ab	0.165 (0.006)a	0.741 (0.03)a	0.110 (0.003)a
Sulfur w/ sticker	1.672 (0.05)abc	0.159 (0.006)a	0.703 (0.03)a	0.114 (0.003)a
Sulfur w/o sticker	1.746 (0.05)c	0.178 (0.006)a	0.715 (0.03)a	0.111 (0.003)a
Sulfur ground	1.7 (0.05)bc	0.169 (0.006)a	0.752 (0.03)a	0.111 (0.003)a
P > F	0.0435	0.114	0.7057	0.0625
	Ca %	**Mn ppm**	**Mg %**	**Na %**
Control	0.389 (0.02)a	563.5 (83.9)a	0.126 (0.008)a	0.050 (0.007)a
Bravo	0.395 (0.02)a	596.2 (83.9)a	0.126 (0.008)a	0.051 (0.007)a
Sulfur w/ sticker	0.414 0.02)a	582.1 (83.9)a	0.125 (0.008)a	0.057 (0.007)a
Sulfur w/o sticker	0.391 (0.02)a	496.5 (83.9)a	0.114 (0.008)a	0.049 (0.007)a
Sulfur ground	0.388 (0.02)a	552.1 (83.9)a	0.133 (0.008)a	0.067 (0.007)a
P > F	0.9419	0.9321	0.5694	0.4157
	B ppm	**Zn ppm**	**Fe ppm**	**Cu ppm**
Control	17.76 (1.08)b	31.1 (4.98)a	66.5 (3.04)a	9.8 (0.50)a
Bravo	23.34 (1.08)a	27.4 (4.98)a	64.3 (3.04)a	9.6 (0.50)a
Sulfur w/ sticker	18.1 (1.08)b	28.9 (4.98)a	66.8 (3.04)a	9.9 (0.50)a
Sulfur w/o sticker	19.16 (1.08)b	40.3 (4.98)a	67.7 (3.04)a	10.1 (0.50)a
Sulfur ground	18.03 (1.08)b	32.4 (4.98)a	65.2 (3.04)a	9.9 (0.50)a
P > F	0.0032	0.4103	0.9388	0.9709

plication treatment had significantly lower infection than the sulfur ground treatment (p-value=.0330), which had the highest level of infection of all treatments. However, the thiolux ground treatment did not differ significantly from the thiolux with-sticker and control treatments (p-values=.4743 and .1954, respectively). The 2000 needle cohort showed no significant differences between treatments (p-value>.05) (Figure 4). However, the Bravo treatment showed a trend of having the least amount of infection when compared to all other treatments.

Sapling Needle Weights

There were no treatment effects on mean weight per 100 oven-dried needles for the 1999 cohort needles sampled during December of 1999 or the 2000 cohort needles sampled in January 2001.

PCR analyses

Bravo was the most effective treatment in reducing the colonization of foliage by *P. gaeumannii* nine months after treatment (Figure 2). The Bravo treatment had a significantly decreased infection level when compared to all other treatments (p-value=.0001). The thiolux with sticker was also significantly effective at lowering SNC infection levels when compared to the control treatment (p-value=.005), but less effective than the Bravo treatment. All other treatments were not significant in lowering SNC infection levels when compared to the control treatment. However, the thiolux with-no-sticker treatment carried only half the infection level of the control treatment.

Based on samples taken in February 2001 (19 months after treatment), the 1999 cohort needles treated with Bravo had significantly lower infection levels than the control treatment (p-value=.0001)(Figure 3). The thiolux foliar ap-

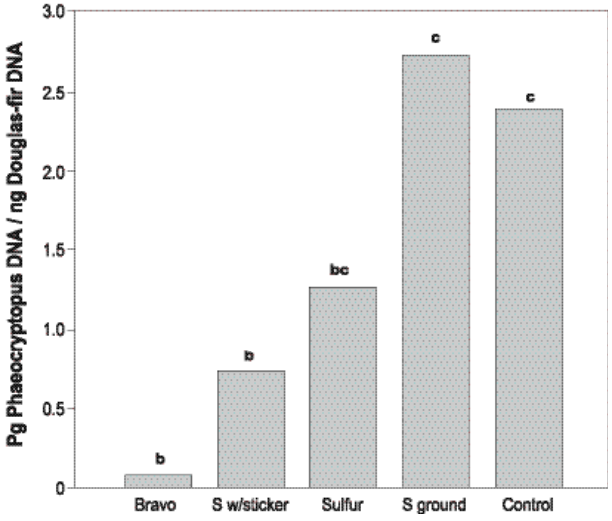


Figure 2. 1999 Polymerase Chain Reaction (PCR) results (pg Phaeocryptopus *DNA / ng Douglas-fir DNA), 1999 needle cohort, sampled April 25, 2000. Bars associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level.*

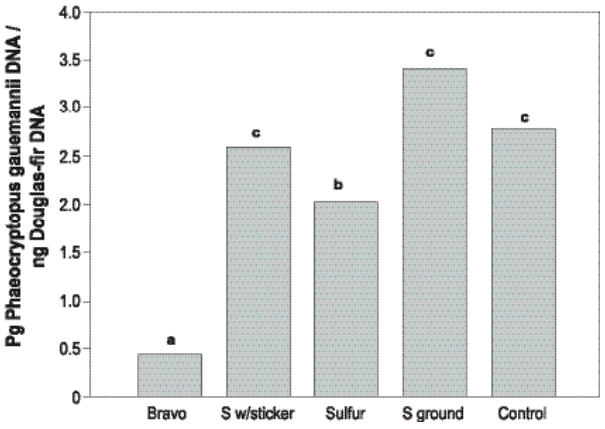


Figure 3. 2000 Polymerase Chain Reaction (PCR) results (pg Phaeocryptopus *DNA / ng Douglas-fir DNA), 1999 needle cohort. sampled Feb. 2001. Bars associated with the same letter are not significantly different at the α≤0.05 level.*

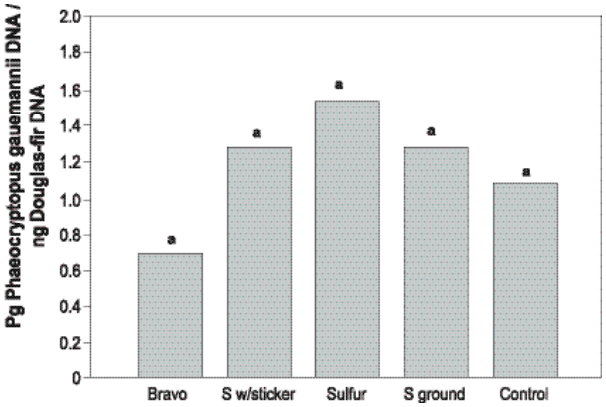


Figure 4. 2000 Polymerase Chain Reaction (PCR) results (pg Phaeocryptopus *DNA / ng Douglas-fir DNA), 2000 needle cohort. Bars associated with the same letter are not significantly different at the α≤0.05 level.*

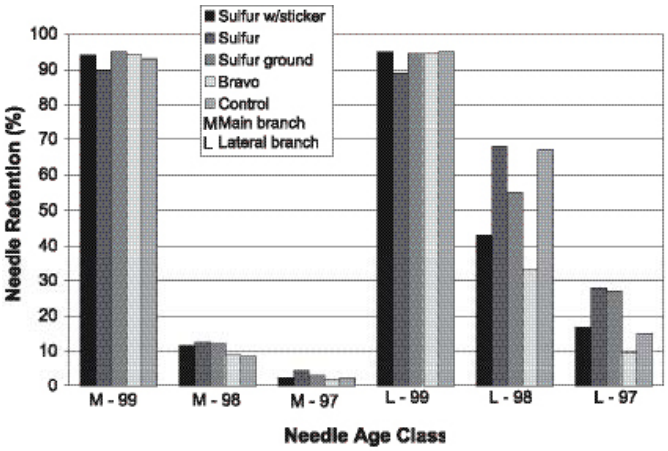


Figure 5. Needle retention assessment by treatment, May 2000.

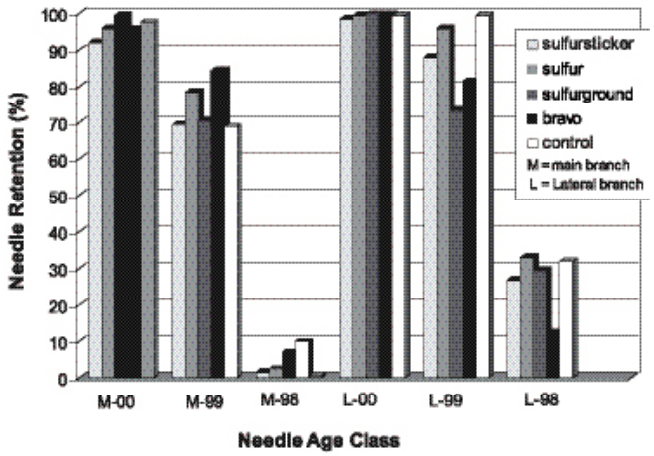


Figure 6. Needle retention assessment by treatment, May 2001.

Needle Retention

The May 2000 needle assessment showed no significant difference between treatments with regard to the retention of the treated 1999 main branch needle cohort (Figure 5; p-value=.4337). Additionally there were no significant differences among the main 1998, main 1997, lateral 1999, lateral 1998, and lateral 1997 branches. A partial explanation for these results is that the majority of needles are not cast until the summer months during the second growing season.

The May 2001 needle assessment showed no significant difference among treatments for any main branches or laterals (Figure 6). However, the treated main 1999 needle cohort showed an approximate increase of 19% needle retention for the bravo treatment when compared to the control treatment.

Discussion

DBH and height growth did not differ significantly among respective treatments after the first or second growing season. Based on infection results this is not surprising. Only the Bravo treatment had a significant impact on decreasing infection. Perhaps if more than one-year worth of treatments were implemented, a difference in growth may have been recognized with a larger increase in photosynthetic area of the tree due to an increase in the retention of needle cohorts. It can be concluded that no treatment had an effect on DBH or height growth after one and/or two full growing seasons.

Based on field trials it appears that Bravo is an effective treatment in reducing *P. gaeumannii* infection levels in Douglas-fir nine months after treatment at a rate of 3.75 pts/100 gallons when sprayed on the foliage three different times at two week intervals during shoot elongation (the highest infection period). This is consistent with published results from studies on SNC disease in Douglas-fir Christmas trees, which have shown Bravo to be effective in reducing infection by *P. gaeumann*ii (Chastagner and Byther 1982).

Thiolux with-sticker also reduced *P. gaeumannii* infection. The amount of *P. gaeumannii* colonization in this treatment group was intermediate between the Bravo and Thiolux with-no-sticker. The amount of *P. gaeumannii* DNA detected was approximately ten times greater in the Thiolux with-sticker treatment than for Bravo. The Thiolux with-no-sticker was intermediate between the Thiolux with-sticker and the control treatment. Thiolux applied to the ground had no effect on reducing colonization by *P. gaeumannii*. Thiolux effectively reduced ascospore germination and hyphal growth of *P. gaeumannii* in culture studies, and therefore it is likely that reduced foliage colonization was due to a contact fungicidal effect (Stone et. al 2000). This is most likely why the thiolux with-sticker was significant in reducing infection and thiolux with-no-sticker was not. In between treatments there was precipitation that could have inhibited the effectiveness of the thiolux with-no-sticker treatment. This precipitation would reduce the amount of active elemental sulfur on the needle surface, thus decreasing the efficacy of the treatment.

The 1999 foliar nutrient results demonstrate that after six months from treatment there is a significantly higher percentage of sulfur on the trees where sticker was used. However, because foliar samples were not washed before nutrient analysis, it is not possible to say if any or all of the sulfur was absorbed into the needle. It may be that there was sulfur residue on the needle surface after this six month time period.

After 19 months the 1999 needle cohort that had been treated with Bravo remained significantly different from the other respective treatments. However, the thiolux with-sticker and thiolux with-no-sticker treatments did not differ significantly from the control after this extended period of time. The reason for this is that despite having infection levels that were significantly reduced when compared to the control treatment, there was fungus that did colonize the needle. This fungus continued to grow throughout the following year, thus increasing its presence within individual needles. Therefore, the significant difference in infection reduction that was seen after the initial nine-month period was not seen after 19 months. This demonstrates the need for further research dealing with rates of elemental sulfur that could potentially carryover significant infection reductions two years after treatment when looking at the same needle cohort.

While there were no significant reductions in fungus within the 2000 needle cohort, there was a trend that showed the Bravo treatment did have reduced levels of *P. gaeumannii* when compared to all other treatments. One explanation for this may be reduced spore loads on the trees that were treated with Bravo due to decreased infection in the previous years needles. It is obvious that the spore load of an individual tree would be reduced, thus decreasing the overall number of spores that were available for infecting the current year's needle cohort. However, because this is a wind-borne fungus it seems as though neighboring trees and stands that were infected would have plenty of spores that would readily infect the untreated 2000 cohort. Future research is needed in determining the rate at which spores travel in order to determine if one year worth of treatment is beneficial in reducing infection over the long term.

There were no significant differences among needle retention two years after the treatments were applied. This is not overly surprising because the majority of needles would tend to be cast during the summer months two years after treatment and the survey was conducted in May (before the next shoot elongation). As presented earlier it was only the bravo treatment that reduced SNC infection significantly. Therefore, one would infer that needle casting would continue at the present rates. However, a trend is developing in that the bravo treatment has increased needle retention of approximately 19% on the main 1999 needle cohort when compared with the control treatment. This gap would be expected to increase over time because the 1999 cohort that was treated with bravo is nearly free of *P. gaeumannii*, while

the control is heavily infected, thus causing casting of this cohort during the summer of 2001. An assessment the following year would validate this assumption.

When looking at both the 2000 and 2001 needle retention figures (see figures 5 and 6) a dramatic difference is seen between the retention of the second year cohort needles over all treatments. The increased retention in the 2001 figure could possibly be attributed to dryer conditions during the period of time where susceptibility to infection was the greatest (June and July 2000). Additionally, mild weather patterns during the spring of 2001 could have potentially slowed the casting of needles until later in the year.

Conclusions and Recommendations

Based on the aforementioned results, the use of Bravo as a means to decrease SNC infection on an individual tree basis is effective. Trends showed that thiolux with-sticker applied as a foliar application was significant in decreasing *P. gaeumannii* in the first growing season. However, the fungus that was able to colonize the needle in the first year (even though it was at a significantly decreased level from the control treatment) continued to grow throughout the following year. This response negated any positive effects from the initial treatment, thus rendering this treatment as an ineffective way to decrease SNC infection for more than one year. More research on rates and timing of applications are needed to determine if thiolux can be used effectively to decrease SNC infection on an individual tree and stand level basis. Opportunities exist to further study the potential nutrient effects of elemental sulfur over an extended period of time.

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Sulfur Fungicide Studies

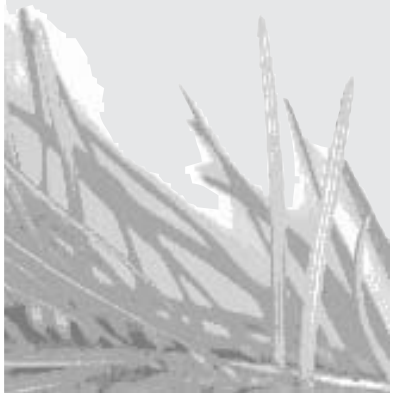
Jeffrey Stone, Paul Reeser, Wendy Sutton, and Gary Chastagner

Abstract

Spring applications of micronized sulfur fungicides were compared with chlorothalonil for control of Swiss needle cast in forest plantations of Douglas-fir. At the highest rates, 30 and 60 lb/ac in broadcast spray applications, or at 15 lb/100 gal in saturation spray applications, Thiolux micronized sulfur with a spreader-sticker reduced foliage infection by *Phaeocryptopus gaeumannii* compared to unsprayed controls at some sites. The addition of a spreader-sticker improved efficacy of Thiolux. Sprays applied later were somewhat more effective in reducing infection than early applications. Thiolux was not as effective as chlorothalonil in reducing infection, even at the highest rates. Results suggest that modifications of rate and time of application may improve efficacy of elemental sulfur for control of Swiss needle cast.

Introduction

Fungicides have proven effective in controlling Swiss needle cast (SNC) in commercial Christmas tree plantations (Skilling 1981, Hadfield and Douglas 1982, Chastagner and Byther 1982, OSU Disease Control Handbook 2001), but to date the use of fungicides to control the disease in forest plantations has been limited. In Christmas tree plantations, chlorothalonil fungicides (Daconil, Bravo) are the most widely used materials and provide good control. Preliminary studies conducted by the Oregon Department of Forestry (A. Kanaskie pers. comm.) also have been successful in reducing Swiss needle cast symptoms in forest plantations with aerial application of Daconil fungicide. Although chlorothalonil is recommended for control of SNC in Christmas trees, the level of control necessary to reduce growth losses due to SNC in forest plantations is not known. Preliminary investigations to identify reduced risk fungicides for efficacy in controlling SNC in forest plantations suggested that micronized sulfur formulations (e.g. Thiolux) could be effective in controlling SNC while presenting relatively low environmental risk (Crane et al. 2000, Stone et al. 2000). In addition to disease control, foliar sulfur applications appeared to increase uptake of some nutrients (Crane et al 2000, Stone unpub.) that may result in increased growth. This study was undertaken to evaluate the efficacy of a micronized sulfur fungicide in contolling SNC in Douglas-fir forest plantations. The objectives were to evaluate application rates and timing of Thiolux fungicide in comparison to chlorothalonil.



Materials and Methods

Study Sites

Duplicate studies were established at two sites in western Oregon and one site in western Washington: Starker Forest, Inc. Bickford, T11S, R8W, S6, Simpson, Tillamook, T2S, R9W, S6, and Rayonier Forest, Humptulips, WA. The study sites were approximately one acre portions of commercial Douglas-fir reforestation plantations approximately 4 years old with trees 2-3 m in height at the time of application. Two studies were conducted at each site, a hand spray study and a broadcast spray study. There was a moderate to high level of disease in each of the study sites.

Hand Spray Study

The objective of this study was to examine the effect of application timing and the addition of Tactic sticker on the effectiveness of Thiolux sulfur in controlling SNC in comparison to chlorothalonil. Fungicide sprays were applied to newly emerged foliage to run off with a back pack sprayer. Thiolux 75W was applied at the rate of 15 pounds per 100 gallons of water, Daconil Weather Stik was applied at 5.5pts/100 gallons. Treatments in this study were:

1. Untreated control
2. Thiolux spray to wet at 15 lb/100 gal, with Tactic sticker, applied when approximately 50% of buds have opened and at least 1″ elongation (first application)
3. Thiolux spray to wet at 15 lb/100gal, with Tactic sticker, applied at 10 days following treatment 2 (second application)
4. Thiolux spray to wet at 15 lb/100 gal, with Tactic sticker, applied on both application dates.
5. Thiolux spray to wet at 15 lb/100gal, without sticker, applied on both application dates.
6. Daconil Weather Stik, spray to wet at 5.5 pt/100 gal on both application dates

The studies were a randomized complete block design with four blocks; each block having six treatments with 10 trees/treatment/block. Treatments were applied on May 22 and June 5, 2000 at the Starker Bickford site, on May 23 and June 15, 2000 at the Simpson Tillamook site, and on May 30 and June 13, 2000 at the Humptulips, WA site.

Broadcast Spray Study

The objective of this study was to examine the effectiveness of broadcast applications of different rates of Thiolux sulfur in controlling SNC in comparison to chlorothalonil. Fungicide sprays were applied to newly emerged foliage at measured rates by means of a boom sprayer equipped with 8003LP nozzles was used to apply all the treatments over the tops of trees at 15 psi. Thiolux with Tactic sticker was applied at the rate of 15, 30 or 60 pounds per acre. Daconil Weather Stik was applied at 5.5 pts per acre. Treatments compared in this study were:

1. Untreated control
2. Thiolux with sticker at 15 lb/acre, applied first when approximately 50% of buds have opened and shoots are at least 1″ long, a second application at ten days following the first application.
3. Thiolux with sticker at 30 lb/ac, applied as for treatment 2.
4. Thiolux with sticker at 60 lb/ac, applied as for treatment 2.
5. Daconil Weather Stik at 5.5 pts. per acre, applied as for treatment 2.

The study was a randomized complete block design with four blocks; each block with five treatments with ten trees/treatment/block. Treatments were applied in the equivalent of 30 gallons of water per acre at the Starker Bickford site on May 22 and June 5, 2000, at the Simpson Tillamook site on May 23 and June 15, 2000, and at the Humptulips, WA site on May 30 and June 13, 2000.

Assessment

Colonization of foliage by *Phaeocryptopus gaeumannii* was assesssed by means of a quantitative polymerase chain reaction assay (QPCR)(Winton et al. in press, Winton et al 2000). For QPCR assessment, two shoots were collected from each tree in Feb, 2001. Needles were stripped from the shoots, placed in labeled envelopes, and stored frozen. A sample of ten needles was drawn from each envelope for DNA extraction and amplification. The amount of *P. gaeumannii* DNA relative to Douglas-fir DNA (pg *P. gaeumannii* per ng Douglas-fir) is given for the Simpson and Humptulips, WA sites. Because of technical problems, the Douglas-fir DNA for the Bickford site was not quantified. Values given for this site only are pg *P. gaeumannii* in the ten-needle sample.

Statsitical Analysis

Statistical analyses were carried out with the General Linear Models procedure in the SYSTAT statistics package (SYSTAT 9.0), with means comparisons by means of Fisher's LSD.

Results

Hand Spray Study

Thiolux fungicide applications reduced foliage colonization by *P. gaeumannii* at the Bickford and Tillamook sites (Figs. 1, 2) but not at the Humptulips site (Fig. 3). At the Bickford and Tillamook sites, the late application or two applications provided better control than a single early application. Daconil Weather Stik was the most effective treatment at all sites. The addition of Tactic spreader-sticker improved control by Thiolux at the Bickford and Tillamook sites. Two applications of Thiolux with Tactic was significantly, although only moderately, different from two applications without the spreader-sticker at the two Oregon sites. None of the Thiolux treatments were significantly different from control at the Humptulips, WA site.

In addition to quantitative PCR, trees at the Humptulips site were also evaluated for incidence of pseudothecia and foliage color in April, 2001 (Table 1). By these measures, only Daconil Weather Stik reduced incidence of pseudothecia, but the early Thiolux application reduced the disease index (incidence x severity). Foliage color was improved by two Thiloux applications regardless of addition of the Tactic sticker. Foliage color rating for two Thiolux applications was not different from the Daconil Weather Stik.

Broadcast Spray Study

Thiolux fungicide applications at the highest rates significantly, although only moderately, reduced foliage colonization by *P. gaeumannii* at the Tillamook and Humptulips sites (Figs 5,6), but not at the Bickford site (Fig. 4). At the Bickford site, quantitative PCR values for Thiolux treatments were not statistically different from unsprayed control.

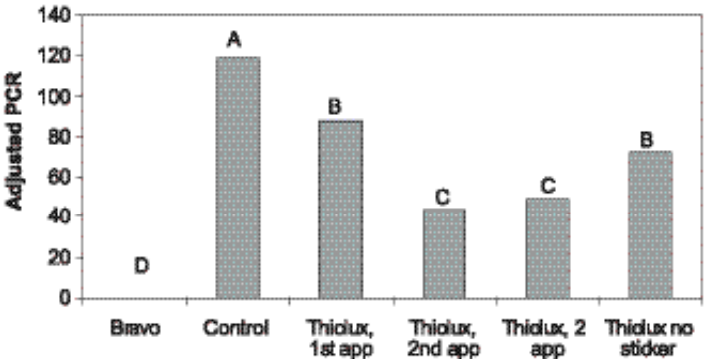


Figure 1. Results of spray to wet fungicide applications on colonization of foliage by P. gaeumannii *at the Bickford site. Adjusted PCR is pg* P. gaeumannii *DNA in a sample of 10 needles as assessed by quantitative PCR. Columns are treatment means for four blocks. Columns with different letters are different at $p < 0.05$.*

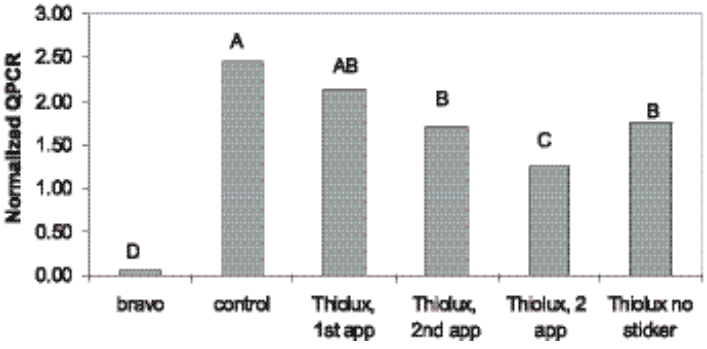


Figure 2. Results of spray to wet fungicide applications on colonization of foliage by P. gaeumannii *at the Tillamook site. Normalized QPCR is pg* P. gaeumannii *DNA per ng Douglas-fir DNA in a sample of 10 needles as assessed by quantitative PCR. Columns are treatment means for four blocks. Columns with different letters are different at $p < 0.05$.*

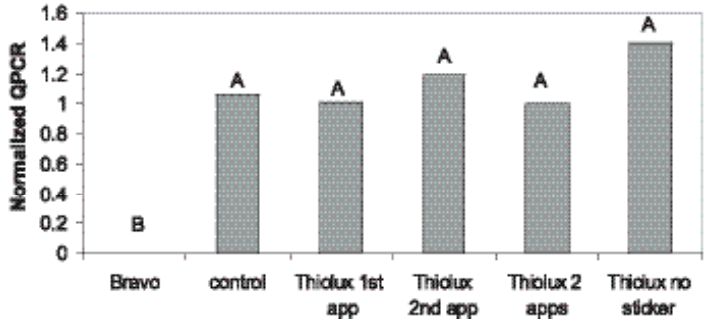


Figure 3. Results of spray to wet fungicide applications on colonization of foliage by P. gaeumannii *at the Humptulips, WA site. Normalized QPCR is pg* P. gaeumannii *DNA per ng Douglas-fir DNA in a sample of 10 needles as assessed by quantitative PCR. Columns are treatment means for four blocks. Columns with different letters are different at $p < 0.05$.*

Table 1. Effectiveness of Thiolux in controlling the development of Swiss needle cast on Douglas-fir when newly emerging shoots are sprayed to wet with a hand sprayer (DF 300).

			Disease[2]		
Treatment[1]	Tactic	Timing	Incidence	Index	Needle color[2]
Check	-	-	9.8 a	20.7 a	2.95 a
Thiolux 80W	+	E	9.5 a	14.0 b	2.55 abc
Thiolux 80W	+	L	9.5 a	19.5 a	2.63 a
Thiolux 80W	+	E + L	9.8 a	18.0 a	2.10 c
Thiolux 80W	-	E + L	9.4 a	19.3 a	2.41 bc
Daconil Weather Stik 720	-	E + L	0.03 b	0.03 c	2.10 c

[1] 15 lbs of Thiolux 80W, Thiolux plus Tactic, or 5.5 pts of Daconil Weather Stik were mixed with 100 gallons of water. Treatments were applied as spray to wet applications on May 30 (E) and/or June 13, 2001 (L). Each treatment was applied to ten trees in each of four blocks. On May 30, 2000 the new growth averaged 3.1 inches in length.

[2] Disease assessments and needle color was evaluated on April 2, 2001. Numbers in columns followed by the same letter are not significantly different, P=0.05.

Treatments with the Tactic spreader-sticker alone, applied at the Bickford and Tiallmook sites only, were not different from unsprayed control. Daconil was very effective in reducing infection at all three sites.

Trees at the Humptulips site were also evaluated for pseudothecia incidence and foliage color in April, 2001 (Table 2). By these measures, only Daconil was effective in reducing incidence of pseudothecia, and disease index (incidence x severity). Foliage color was equivalent for all treatments and unsprayed control.

Discussion

Thiolux micronized sulfur fungicide was moderately effective in reducing colonization of Douglas-fir foliage by *P. gaeumannii* in both saturation and broadcast spray applications, but not at all sites. Results for both types of application suggest that higher rates, multiple applications, and addition of a spreader-sticker improve fungicide efficacy. In the saturation spray study where only one application was made, it appears that the later application was more effective in reducing *P. gaeumannii* infection than the early application. Evaluation of foliage color for trees at the Humptulips site indicate that higher application rates were not phytotoxic.

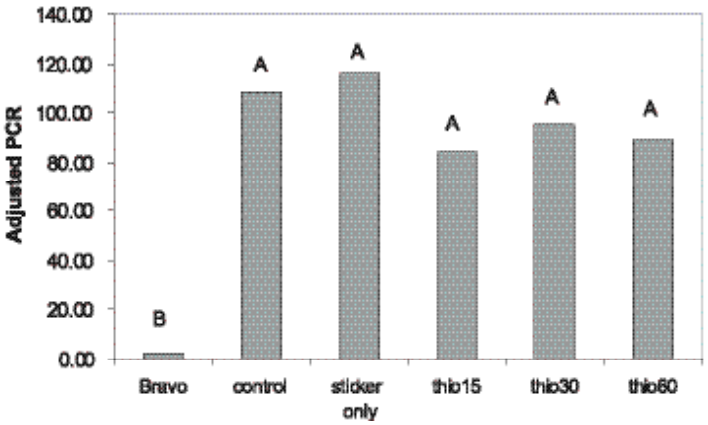


Figure 4. Results of broadcast spray application of Thiolux sulfur with Tactic spreader-sticker and Daconil Weather Stik fungicides on colonization of foliage by Phaeocryptopus gaeumannii *at the Starker Forests Bickford site. Adjusted PCR is pg of* P. gaeumannii *DNA in a sample of 10 needles. Columns are treatment means for four blocks. Columns with different letters are different at p < 0.05.*

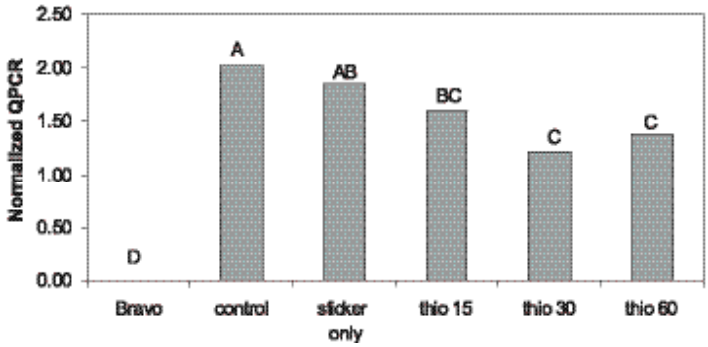


Figure 5. Results of broadcast spray application of Thiolux sulfur with Tactic spreader-sticker and Daconil Weather Stik fungicides on colonization of foliage by Phaeocryptopus gaeumannii *at the Simpson Tillamook site. Normalized QPCR is pg of P. gaeumannii DNA per ng Douglas-fir DNA in a sample of 10 needles. Columns are treatment means for four blocks. Columns with different letters are different at p < 0.05.*

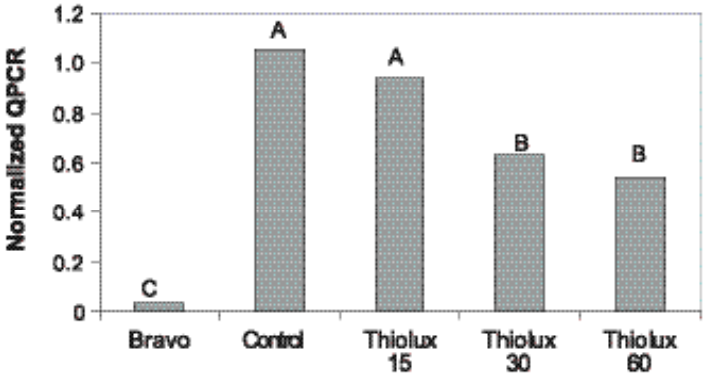


Figure 6. Results of broadcast spray application of Thiolux sulfur with Tactic spreader-sticker and Daconil Weather Stik fungicides on colonization of foliage by Phaeocryptopus gaeumannii *at the Rayonier Humptulips site. Normalized QPCR is pg of* P. gaeumannii *DNA per ng Douglas-fir DNA in a sample of 10 needles. Columns are treatment means for four blocks. Columns with different letters are different at p < 0.05.*

Table 2. Effectiveness of broadcast applications of Thiolux and Daconil Weather Stik in controlling the development of Swiss needle cast on Douglas-fir (DF 400).

		Disease[2]		
Treatment[1]	Rate/acre	Incidence	Index	Needle color[2]
Check	-	9.5 a	19.6 a	2.7 a
Thiolux 80W	15 lbs	8.9 a	18.6 a	2.5 a
Thiolux 80W	30 lbs	9.1 a	16.2 a	2.5 a
Thiolux 80W	60 lbs	8.5 a	16.0 a	2.2 a
Daconil Weather Stik 720	5.5 pt	0.4 b	0.4 b	1.9 a

[1] Treatments were applied as broadcast boom application over the top of the trees at the rate of 30 gallons per acre. Treatments were applied to ten trees in each of four blocks on May 30 and June 13, 2000. The new growth averaged 3.1 inches in length on May 30th.

[2] Disease assessments and needle color was evaluated on April 2, 2001. Numbers in columns followed by the same letter are not significantly different, P=0.05.

Possible explanations for inconsistent results among sites might be differences in the timing of applications or differences in initial disease levels at the sites. Sprays were applied at the Humptulips site beginning one week later than the two Oregon sites, although the late applications were similar. Although there was no difference between one and two applications at the Humptulips site, the later initial spray and shorter duration between sprays in the broadcast study may have contributed to the greater control observed at the Humptulips site compared to the Bickford and Tillamook sites. Initial disease levels also appeared to be somewhat lower at the Humptulips site than either the Bickford or Tillamook sites, which might have contributed to a better level of control.

Although sulfur fungicide tested here does not provide a level of control comparable to chlorothalonil, the level of control sufficient to manage SNC in forest plantations is not known. Our results suggest that modifications of application rate and timing might improve control of SNC by sulfur fungicides. Furthermore, the potential of micronized elemental sulfur to promote growth as a nutrient in forest plantations in the Coast Range has not yet been addressed. Given its relatively low environmental and toxicological risk and moderate level of disease control, elemental sulfur fungicide remain a promising option for fungicidal SNC control. Further studies should be undertaken to determine whether a sufficient level of control can be achieved to obtain an economically justified growth improvement.

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Fungicidal Control of Swiss Needle Cast in Stands of Douglas-fir Timber

Gary Chastagner and Jeff Stone

Abstract

During this past year, the effectiveness of two reduced risk fungicides, two formulations of chlorothalonil, and two sulfur products in protecting needles from Swiss needle cast infection were evaluated. The results from traditional springtime pseudothecial based disease assessments from these trials were also compared to PCR data from samples collected in February. Results indicate that: 1) Terraguard and Vangard reduced risk fungicides are not effective against SNC; 2) Daconil Weather Stik is more effective than Daconil Ultrex when the foliage is sprayed to wet; 3) there was a clear trend of decreasing disease with increasing rates of Thiolux sulfur when treatments were applied early and/or late; 4) results with Golden Dew sulfur were much more variable than with Thiolux; and 5) there was a very high correlation between pseudothecial based disease assessments in April and PCR data from February.

Progress Report

When this project was initiated in 1998, it had two objectives: 1) to identify fungicides that are effective in controlling Swiss needle cast (SNC) when applied as protectants in the spring and 2) determine the effectiveness of fall applications of selected fungicides in disrupting inoculum production and reducing subsequent disease development.

During this past year the focus of this project has been to evaluate the effectiveness of two reduced risk fungicides and various sulfur products in protecting needles from infection (Table 1). In addition to the traditional springtime disease assessments based on pseudothecia, samples were also collected in February for PCR analysis at Oregon State University.

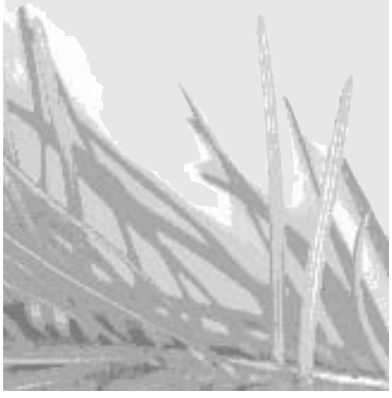

Table 1. Products included in 2000/2001 fungicide protectant trials.

Trade name and formulation	Active ingredient
Daconil Weather Stik 720	chlorothalonil
Daconil Ultrex 82.5WDG	chlorothalonil
Golden Dew Sulfur 92%	sulfur
Tactic sticker	synthetic latex and organosilicone
Terraguard 50W	triflumizole
Thiolux 80W	sulfur
Vangard 75W	cyprodinil

No additional inoculum disruption work was done during 2000/2001. Although fall applications of systemic fungicides disrupted the development of pseudothecia and reduced the production of ascospore inoculum at inland test sites, none

of the treatments were effective in reducing inoculum levels at sites along the coast, which produced very high levels of inoculum (see last year's annual report).

2000/2001 Studies

The following trials were established during 2000 in timber stands along the Washington coast. Unless stated otherwise, all treatments were applied with a Solo backpack sprayer that was equipped with an 8003LP nozzle at 15 psi.

Reduced risk fungicide test (DF 200) –The efficacy of two reduced risk fungicides, Terraguard and Vangard, in protecting new growth from SNC was compared to Daconil Weather Stik. Treatments were applied on May 30, 2000. Each treatment was applied to a single tree in each of five blocks by spraying the new growth to wet. Disease and needle color were assessed as indicated below on samples collected on April 2, 2001. PCR testing was also done on samples collected on February 21, 2001.

Chlorothalonil application method, rate and formulation test (DF 500) – This test was conducted to determine the effect application method and rate had on the control of SNC with a single application of two formulations of chlorothalonil (Daconil Ultrex and Daconil Weather Stik). The high and low label rates of each product were used and the applications consisted of either mixing the product in 100 gallons of water and spraying the foliage to wet or mixing the product in 30 gallons of water and applying with a broadcast boom over the top of the trees at the rate of 30 gallons per acre. Each treatment was applied to two trees in each of ten blocks on May 30, 2000. Disease and needle color were assessed as indicated below on samples collected on April 2, 2001. PCR testing was also done on samples collected on February 21, 2001.

Sulfur Tests

Last year, preliminary data from a series of industry trials in Oregon indicated that it might be possible to reduce the development of SNC with spring foliar applications of Thiolux. Laboratory spore germination tests also showed that Thiolux and Golden Dew sulfur were equally effective in reducing germination and inhibiting growth of SNC ascospore germ tubes. As a result of these field and laboratory tests, three field trials were established in 2000 to further examine the potential of using foliar sulfur applications in controlling SNC and obtain a better understanding of the effect of rates and application timing/method on the extent of disease control. Two of the trials were duplications of studies that Oregon State University established at test sites in Oregon.

Oregon State University spray to wet Thiolux test (DF 300) – This test examined the effect of application timing and the addition of Tactic sticker on the effectiveness of Thiolux sulfur in controlling SNC when the new growth was sprayed to wet with a backpack sprayer. Thiolux 75W was applied at the rate of 15 pounds per 100 gallons of water. Some Thiolux treatments contained Tactic (5%). Daconil Weather Stik at 5.5pts/100 gallons was included as one of the treatments. All treatments were applied on May 30, 2000 and some treatments included an additional application on June 13, 2000. Disease and needle color were assessed as indicated below on samples collected on April 2, 2001. PCR testing was also done on samples collected on February 21, 2001.

Oregon State University broadcast Thiolux test (DF 400) – This test examined the effectiveness of broadcast applications of different rates of Thiolux sulfur in controlling SNC. A boom equipped with 8003LP nozzles was used to apply all the treatments over the tops of trees at 15 psi. Thiolux was applied at the rate of 15, 30 or 60 pounds per acre. Tactic spreader was used with all of the Thiolux treatments. Daconil Weather Stik at 5.5 pts per acre was included as a one of the treatments. All the treatments were applied in the equivalent of 30 gallons of water per acre on May 30 and June 13, 2000. Disease and needle color were assessed as indicated below on samples collected on April 2, 2001. PCR testing was also done on samples collected on February 21, 2001.

Thiolux/Golden Dew sulfur test (DF 600) - The third trial was designed to examine the effect of sulfur product (Thiolux and Golden Dew), rate (15, 30, 60 and 90 lbs), spray coverage (spray to wet the foliage or broadcast on a per acre basis) and timing (early, late, or early and late) on the control of SNC. Daconil Weather Stik at 5.5 pts was also included as one of the treatments. All the treatments were applied in the equivalent of 100 gallons (spray to wet) or 30 gallons of water per acre (broadcast) on May 30

and/or June 13, 2000. Each treatment was applied to a single tree in each of eight blocks. Disease and needle color were assessed as indicated below on samples collected on April 2, 2001. PCR testing was also done on samples collected on February 21, 2001.

Washington SNC impact studies (DF 399A & B) – During early spring 1999, stands of Douglas-fir timber along the central Washington coast exhibited extensive needle discoloration and premature needle loss compared to what has been seen along the Oregon coast. In an effort to obtain some information on the role SNC has on the condition of the trees in these stands, paired fungicide control plots were installed at two sites in 1999. Applications of Daconil Weather Stik 720 (5.5 pts/100 gallons) were applied to half of the trees at each site using a high-pressure sprayer. At the Rayonier site (DF 399A), all of the trees within one of five 60′ X 100′ paired plots were sprayed. At the Grays Harbor County site (DF 399B), there are three replications of ten treated and check trees. During spring 2000, additional Daconil treatments were applied to the trees that had been treated in 1999. Samples of 1999 and 2000 growth were collected from each tree in these plots on April 4, 2001 and rated for disease, needle color and needle loss. PCR testing was done for samples of 2000 shoots only. Shoots were collected from all the trees at the Grays Harbor site and 10 randomly selected trees in each 60′ X 100′ plot at the Rayonier site. All the PCR samples were collected on February 22, 2001.

PCR Assessments

Relationship between pseudothecia assessments and PCR data – We have routinely used a combination of visual pseudothecia assessments to determine the effectiveness of various fungicidal treatments in controlling SNC. As indicated below, these assessments include disease incidence and severity, which are then used to calculate a disease index. During this past year, a combination of our standard pseudo-thecia and PCR assessments were done in an effort to examine the relationship between this new molecular approach and our traditional pseudothecia assessments of SNC.

For this study, a set of paired 2000 shoots was tagged on each tree. One of the shoots from each pair was removed on February 21 or 22, 2001 and shipped to Oregon State University for PCR analysis. The other shoot on each tree was harvested on April 2 or 4, 2001 and used for our standard pseudothecia assessments. Linear regression analysis was conducted to determine if there was a correlation between the PCR data and the disease incidence, severity and index data.

Assessment Methods and Scales

Disease Incidence - Number of needles out of ten that have one or more SNC pseudothecia on them.

Disease Severity – Disease severity is rated on a 0 to 6 scale where 0 = none, 1 = <1%, 2 = 1 – 10%, 3 = 11-25%, 4 = 26-50%, 5 = 51-75%, and 6 = >75% of stomates on the needles are plugged with pseudothecia. These ratings are made with the aid of a card that illustrates sections of needles with 180 stomates that have 1, 10, 25, 50, and 75% of the stomates plugged with pseudothecia.

Disease Index - A disease index is calculated by multiplying the disease incidence times disease severity. Thus our disease index ranges from 0 to 60.

Needle Loss – Needle loss is rated on a scale of 0 to 10, where 0 = none, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%,…, and 10 = 91-100% loss.

Needle Color – Needle color is rated on a 1 to 6 scale, where 1 = healthy-appearing dark green needles, 2 = healthy-appearing green needles, 3 = needles with a slight yellow mottling on a green background that may also have brown spots or tips on the needles, 4 = dull green needles with moderate chlorosis that may also have brown spots or tips on the needles, 5 = extensive yellowing/browning, and 6 = uniformly yellow needles that may have some brown spots or tips.

Results from 2000/2001 trials

Reduced risk fungicide test (DF 200) – Shoot lengths at the time of application averaged 3.5 inches. Although Daconil Weather Stik significantly reduced the amount of SNC, applications of the two reduced risk fungicides had no effect on disease development (Table 2). None of the treatments had any effect on needle color (data not shown). Based on these results, there is no indication that Terraguard or Vangard have any

Table 2. Effectiveness of two reduced risk fungicides in controlling Swiss needle cast on Douglas-fir (DF 200).

Treatment[1]	Product/ 100 gal	Disease[2] Incidence	Index	Adjusted PCR[2]
Check	-	10.0 a	28.0 a	1.99 a
Terraguard 50W	4.0 oz	9.8 a	21.8 a	1.56 a
Terraguard 50W	8.0 oz	10.0 a	22.0 a	1.24 a
Terraguard 50W	16.0 oz	10.0 a	24.0 a	1.78 a
Vangard 75W	5.0 oz	8.6 a	22.0 a	1.43 a
Vangard 75W	10.0 oz	9.6 a	24.8 a	1.52 a
Vangard 75W	20.0 oz	8.4 a	17.8 a	1.22 a
Daconil Weather Stik 720	5.5 pt	1.2 b	1.2 b	0.02 b

[1] Treatments were applied with a backpack sprayer on May 30, 2000 when the new growth averaged 3.5 inches in length. Trees were sprayed to wet and each treatment was applied to a single tree in each of five blocks.

[2] Disease incidence and severity data are for April 2, 2001 and PCR data are for February 21, 2001. Numbers in columns followed by the same letter are not significantly different, P=0.05, DMRT.

potential to control SNC.

Chlorothalonil spray coverage, rate and formulation test (DF 500) – Shoot lengths at the time of application averaged 3.1 inches. Overall, the broadcast applications of chlorothalonil resulted in significantly less disease than the spray to wet applications (Table 3). Both formulations of chlorothalonil significantly reduced the level of SNC compared to the non-sprayed checks, but applications of Daconil Weather Stik provided significantly better control than Daconil Ultrex with spray to wet applications (Table 4). None of the treatments had any effect on needle color (data not shown). This test indicates that the Daconil Weather Stik formulation of chlorothalonil is potentially more effective in controlling SNC than Daconil Ultrex with spray to wet applications. Although rate had no effect on the level of control in this test, previous testing with Daconil Weather Stik has shown that the high-label rate is often more effective than the low-label rate under high disease pressure.

Oregon State University spray to wet Thiolux test (DF 300) – Shoot lengths at the time of application on May 30th averaged 3.1 inches. None of the Thiolux treatments had any effect on the incidence of needles with pseudothecia (Table 5). A single early application of Thiolux plus Tactic caused a small, but significant reduction in the overall disease index but not PCR values (Table 5). Applications of Daconil Weather Stik were highly effective in controlling SNC and the needles on trees that were treated with Daconil and multiple applications of Thiolux had slightly better color than the non-sprayed check (Table 5).

Table 3. Effect of application method on the efficacy of Daconil Weather Stik and Daconil Ultrex in controlling the development of Swiss needle cast on Douglas-fir (DF 500).

Application method[1]	Disease[2] Incidence	Index	Adjusted PCR
Spray to wet	3.0 a	3.7 a	.132 a
Broadcast	1.0 b	1.0 b	.060 b

[1] Spray to wet applications consisted of mixing product in 100 gallons of water and spraying the foliage to wet while broadcast applications consisted of mixing the product in 30 gallons of water that was applied as a broadcast boom application over the top of the trees at the rate of 30 gallons per acre. Treatments were applied to two trees in each of ten blocks on May 30, 2000 when the new growth averaged 3.1 inches in length.

[2] Disease incidence and index data are for April 2, 2001 and PCR data are for February. Numbers in columns followed by the same letter are not significantly different, P=0.05.

Oregon State University broadcast Thiolux test (DF 400) – Shoot lengths at the time of application on May 30th averaged 3.1 inches. Although there was a trend for decreasing disease, none of the Thiolux treatments had a significant effect on the incidence of needles with pseudothecia or disease index (Table 6). Increasing rates of Thiolux did result in significantly lower PCR values. Applications of Daconil provided excellent disease control. None of the treatments had any effect on needle color (data not shown).

Thiolux/Golden Dew sulfur test (DF 600) – Application timing and sulfur product had an effect on SNC disease ratings during this trial (Figure 1). The similarity in the disease index and PCR data is readily apparent in Figures 1 and 2. Although there appeared to be a trend of decreasing disease with increasing rates of Golden Dew sulfur, none of the early application treatments except Daconil had any significant effect on SNC. When treatments were applied early and/or late, there was a clear trend of decreasing disease with increasing rates of Thiolux sulfur. The results with Golden Dew applications were much more variable and this product does not appear to be as effective as Thiolux. Applications of Daconil provided very effective disease control irrespective of application timing. Application method generally had no effect on the level of control obtained with any of the treatments.

Based on the results of these sulfur trials, additional research is clearly needed to further determine

Table 4. Effectiveness of two formulations of chlorothalonil in controlling the development of Swiss needle cast on Douglas-fir (DF 500).

	Spray to wet applications				Broadcast applications		
		Disease[2]			Disease[2]		
Treatment[1]	Rate	Incidence	Index	Adj. PCR	Incidence	Index	Adj. PCR
Check	-	9.6 a	19.9 a	0.862 a	9.4 a	22.3 a	0.871 a
Daconil Ultrex 82.5WDG	2.5 lb	4.8 b	5.7 b	0.237 b	1.5 b	1.5 b	0.131 b
Daconil Ultrex 82.5WDG	5 lb	5.3 b	7.1 b	0.171 b	0.4 b	0.4 b	0.333 b
Daconi Weather Stik 720	2.75 pt	1.6 c	1.6 c	0.080 b	1.0 b	1.0 b	0.041 b
Daconil Weather Stik 720	5.5 pt	0.5 c	0.5 c	0.039 b	1.2 b	1.2 b	0.040 b

[1] Data are averages for treatments that were applied as spray to wet applications in 100 gallons of water or broadcast applications that were applied as a boom application over the top of the trees at the rate of 30 gallons per acre. Treatments were applied to two trees in each of ten blocks on May 30, 2000 when the new growth averaged 3.1 inches in length.

[2] Disease incidence and index data are for April 2, 2001 and PCR data are for February 21, 2001. Numbers in columns followed by the same letter are not significantly different, P=0.05.

Table 5. Effectiveness of Thiolux in controlling the development of Swiss needle cast on Douglas-fir when newly emerging shoots are sprayed to wet with a hand sprayer (DF 300).

			Disease[2]			
Treatment[1]	Tactic	Timing	Incidence	Index	Adjusted PCR	Needle color[2]
Check	-	-	9.8 a	20.7 a	1.064 a	2.95 a
Thiolux 80W	+	L	9.5 a	19.5 a	1.193 a	2.63 a
Thiolux 80W	-	E + L	9.4 a	19.3 a	1.405 a	2.41 bc
Thiolux 80W	+	E + L	9.8 a	18.0 a	1.001 a	2.10 c
Thiolux 80W	+	E	9.5 a	14.0 b	1.015 a	2.55 abc
Daconil Weather Stik 720	-	E + L	0.03 b	0.03 c	0.005 b	2.10 c

[1] 15 lbs of Thiolux 80W, Thiolux plus Tactic, or 5.5 pts of Daconil Weather Stik were mixed with 100 gallons of water. Treatments were applied as spray to wet applications on May 30 (E) and/or June 13, 2001 (L). Each treatment was applied to ten trees in each of four blocks. On May 30, 2000 the new growth averaged 3.1 inches in length.

[2] Disease incidence, index and needle color data are for April 2, 2001 and PCR data are for February. Numbers in columns followed by the same letter are not significantly different, P=0.05.

Table 6. Effectiveness of broadcast applications of Thiolux and Daconil Weather Stik in controlling the development of Swiss needle cast on Douglas-fir (DF 400).

		Disease[2]		
Treatment[1]	Rate/acre	Incidence	Index	Adjusted PCR[2]
Check	-	9.5 a	19.6 a	1.1 a
Thiolux 80W	15 lbs	8.9 a	18.6 a	0.9 ab
Thiolux 80W	30 lbs	9.1 a	16.2 a	0.8 ab
Thiolux 80W	60 lbs	8.5 a	16.0 a	0.5 b
Daconil Weather Stik 720	5.5 pt	0.4 b	0.4 b	0.0 c

[1] Treatments were applied as broadcast boom application over the top of the trees at the rate of 30 gallons per acre. Treatments were applied to ten trees in each of four blocks on May 30 and June 13, 2000. The new growth averaged 3.1 inches in length on May 30th.

[2] Disease incidence and index data are for April 2, 2001 and PCR data are for February 21, 2001. Numbers in columns followed by the same letter are not significantly different, P=0.05.

the effect that variables such as formulation, timing and rate have on the effectiveness of foliar applications of sulfur in controlling SNC in timber stands along the coast.

Washington SNC impact studies (DF 399A & B) - At the Grays Harbor test site, virtually no disease was found on the 1999 needles that had been sprayed with Daconil in 1999 and 2000 (Table 7). There was approximately a 3-fold increase in the disease index on the 1999 needles on the non-sprayed trees between the time evaluations were done in 2000 and when they were done in 2001. The 1999 needles that had been sprayed with Daconil also tended to have better color and better retention than needles on trees that were not sprayed (Table 7). Daconil sprays were also very effective in controlling disease development on the 2000 needles, but they had no effect on color and needle loss (Table 8).

Similar results were obtained at the Rayonier test site (Tables 9 & 10). Trees sprayed with Daconil in 1999 and 2000 had significantly lower levels of disease, better needle color and less needle loss than trees that were not sprayed.

PCR Assessments

Relationship between pseudothecia assessments and PCR data –Regression analysis indicated that there was a highly significant correlation between the PCR data and our standard pseudothecia assessments for each of our trials (Table 11). The results of these trials indicate that it is possible to use the PCR test procedure to assess the effectiveness of various fungicidal treatments in controlling SNC.

Although probability values for correlation coefficients were virtually the same for the PCR data and disease incidence, severity and index assessments, additional studies are needed to determine if similar relationships exist over a broader range of disease levels than occurred in these studies. With higher disease levels, it is likely that there would be a higher correlation between disease severity and the disease index than with disease incidence.

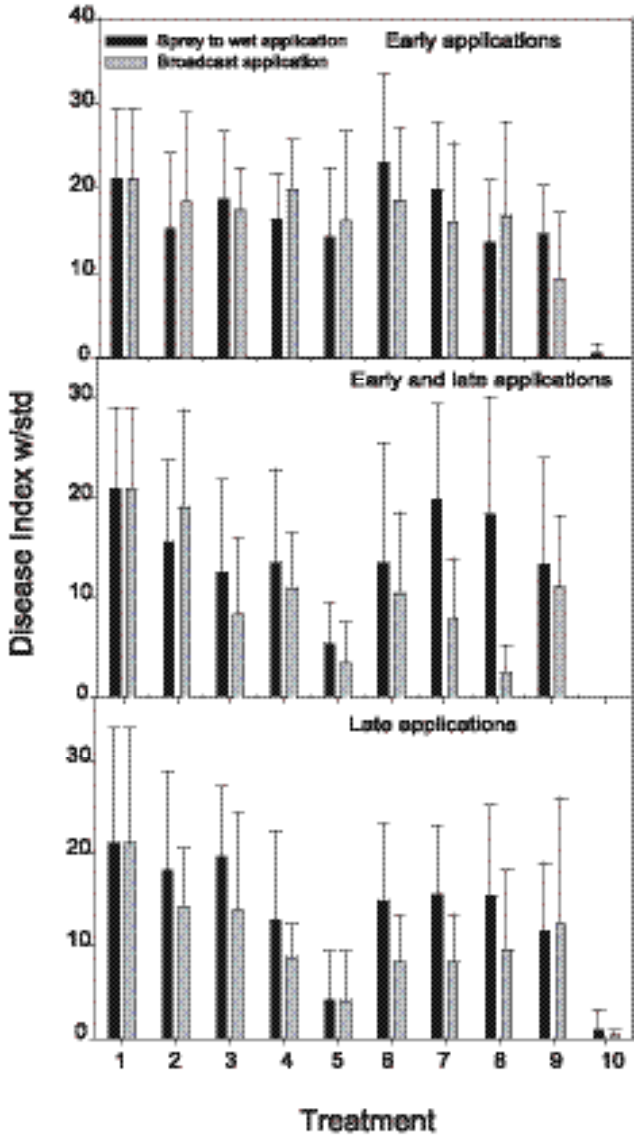

Figure 1. *Effect of applications of Thiolux, Golden Dew, and Daconil on Swiss needle cast disease index ratings. Treatments are: 1. Check, 2. Thiolux 80W (15 lb), 3. Thiolux 80W (30 lb), 4. Thiolux 80W (60 lb), 5. Thiolux 80W (90 lb), 6. Golden Dew 92% (13 lb), 7. Golden Dew 92% (26 lb), 8. Golden Dew 92% (52 lb), 9. Golden Dew 92% (78 lb), and 10. Daconil Weather Stik (5.5 pt).*

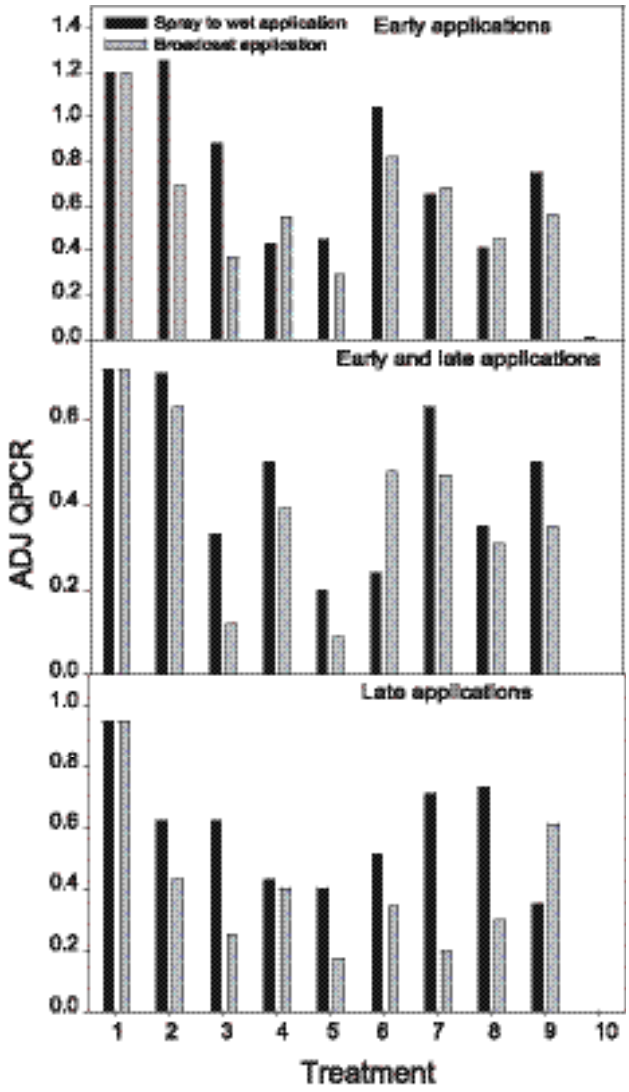

Figure 2. *Effect of applications of Thiolux, Golden Dew, and Daconil on adjusted QPCR values. Treatments are: 1. Check, 2. Thiolux 80W (15 lb), 3. Thiolux 80W (30 lb), 4. Thiolux 80W (60 lb), 5. Thiolux 80W (90 lb), 6. Golden Dew 92% (13 lb), 7. Golden Dew 92% (26 lb), 8. Golden Dew 92% (52 lb), 9. Golden Dew 92% (78 lb), and 10. Daconil Weather Stik (5.5 pt).*

Table 7. Effect of high pressure ground based applications of Daconil Weather Stik 720 in 1999 and 2000 on the development of Swiss needle cast on 1999 needles at the Grays Harbor test site (DF 399B)[1].

Treatment	Prod./ 100 gal	Disease index 2000	Disease index 2001	Needle color 2000	Needle color 2001	Needle loss 2000	Needle loss 2001
Check	-	16.2 a[2]	42.8 a	3.0 a	1.8 a	2.0 a	1.4 a
Daconil Weather Stik 720	5.5 pt	0.0 b	0.1 b	1.8 b	1.3 a	1.5 b	0.7 b

[1] Trees were sprayed to drip on June 11, 1999 and May 25, 2000. Disease and needle color/loss data were taken on April 6, 2000 and April 4, 2001.

[2] Numbers in columns followed by the same letter are not significantly different, P=0.05, t-test.

Table 8. Effect of high pressure ground based applications of Daconil Weather Stik 720 in 2000 on the development of Swiss needle cast on 2000 needles at Grays Harbor test site (DF 399B)[1].

Treatment	Prod./ 100 gal	Disease: Incidence	Disease: Index	Adjusted PCR	Needle: Color	Needle: Loss
Check	-	8.8 a[2]	18.8 a	0.409 a	1.6 a	0.2 a
Daconil Weather Stik 720	5.5 pt	0.3 b	0.1 b	0.026 b	1.1 a	0.2 a

[1] Trees were sprayed to drip on May 25, 2000. Disease incidence, index, and needle color/loss data are for April 4, 2001 and PCR data are for February 22, 2001.

[2] Numbers in columns followed by the same letter are not significantly different, P=0.05, t-test.

Table 9. Effect of high pressure ground based applications of Daconil Weather Stik 720 in 1999 and 2000 on the development of Swiss needle cast on 1999 needles at the Rayonier test site (DF 399A)[1].

		Disease index		Needle color		Needle loss	
Treatment	Prod./100 gal	2000	2001	2000	2001	2000	2001
Check	-	15.2 a[2]	45.2 a	3.5 a	3.2 a	0.3 a	2.0 a
Daconil Weather Stik 720	5.5 pt	0.1 b	0.1 b	2.1 b	1.7 b	0.1 b	0.3 b

[1] Trees were sprayed to drip on June 11, 1999 and May 25, 2001. Disease and needle color/loss data were taken on April 6, 2000 and April 4, 2001.

[2] Numbers in columns followed by the same letter are not significantly different, P=0.05, t-test.

Table 10. Effect of high pressure ground based applications of Daconil Weather Stik 720 in 2001 on the development of Swiss needle cast on 2000 needles at the Rayonier test site (DF 399A)[1].

		Disease			Needle	
Treatment	Prod./100 gal	Incidence	Index	Adj. PCR	Color	Loss
Check	-	8.3 a[2]	12.2 a	0.409 a	2.5 a	0.2 a
Daconil Weather Stik 720	5.5 pt	0.0 b	0.0 b	0.026 b	1.8 b	0.1 a

[1] Trees were sprayed to drip on May 25, 2000. Disease incidence, index, and needle color/loss data are for April 4, 2001 and PCR data are for February 22, 2001.

[2] Numbers in columns followed by the same letter are not significantly different, P=0.05, t-test.

Table 11. Results of linear regression analysis of correlations between April pseudothecia assessments and February PCR data[1]

Test	Data	N	Incidence[2]	Severity[3]	Index[4]
DF 399A	Trt/block values	99	0.661*	0.712*	0.708*
	Treatment averages	10	0.889*	0.934*	0.956*
DF 399B	Trt/block values	58	0.570*	0.673*	0.667*
	Treatment averages	6	0.837 (P=0.038)	0.901 (P=0.014)	0.855 (P=0.030)
DF 200	Trt/block values	40	0.610*	0.558*	0.609*
	Treatment averages	8	0.932*	0.923*	0.965*
DF 300	Trt/block averages	24	0.722*	0.768*	0.768*
	Treatment averages	6	0.940 (P=0.005)	0.949 (P=0.004)	0.935 (P=0.006)
DF 400	Trt/block averages	20	0.739*	0.839*	0.847*
	Treatment averages	5	0.910 (P=0.031)	0.930 (P=0.022)	0.948 (P=0.014)
DF 500	Trt/block averages	99	0.706*	0.718*	0.769*
	Treatment averages	10	0.949*	0.987*	0.989*
DF 600	Overall Trt averages	57	0.734*	0.775*	0.789*
	Early trt. avg	19	0.651 (P=0.003)	0.661 (P=0.002)	0.664 (P=0.002)
	Late trt avg	19	0.765*	0.861*	0.884*
	E & L trt avg	19	0.824*	0.840*	0.843*

[1] Coefficient of correlation and estimated "P" values, * P<0.001

[2] Disease Incidence - Number of needles out of ten that have one or more pseudothecia on them.

[3] Disease Severity – Disease severity is rated on a 0 to 6 scale where 0 = none, 1 = <1%, 2 = 1 – 10%, 3 = 11-25%, 4 = 26-50%, 5 = 51-75%, and 6 = >75% of stomates on the needles are plugged with pseudothecia.

[4] Disease Index - The disease index is calculated by multiplying the disease incidence times disease severity. It can range from 0 to 60.

2001/2002 Trials

One additional sulfur trial was established this year in an effort to confirm the effectiveness of sulfur in controlling SNC. Applications of Thiolux 80W at 90 pounds and Golden Dew at 78.3 pounds, along with Daconil Weather Stik 720 at 5.5 pts were applied to trees near Humptulips, WA. Tactic spreader was added to all of the sulfur treatments at the rate of 8 oz per 100 gallons of water. All treatments were applied as spray to wet applications in 100 gallons of water and as broadcast applications in 30 gallons of water per acre. Non-sprayed trees served as checks. Additional applications of Daconil Weather Stik were also applied to all of the trees that were sprayed in 1999 and 2000 at the two SNC impact test sites during June 2001. Disease and foliage assessments will be made during spring 2002.

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Influence of Bravo fungicide applications on wood properties of Douglas-fir

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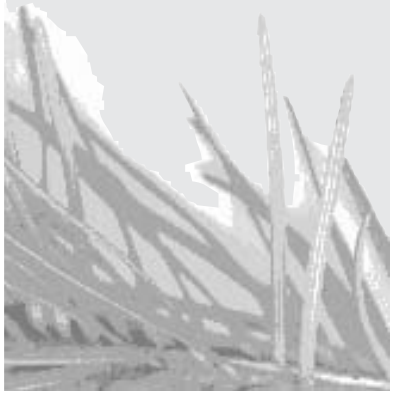
Abstract

The moisture content of sapwood in trees sprayed for 5 years with Bravo was significantly higher than unsprayed trees. We hypothesize that this is a function of the trees' overall poor health in that they did not have sufficient energy (photosynthate) to overcome air embolisms in the tracheids. The reduction in moisture content will result in green logs weighing less from the unsprayed plots than the sprayed plots, even though the dry weight of the loads would not differ. The sprayed trees also showed increased growth, decreased latewood proportion and decreased wood density.

The Oregon Department of Forestry established a trial near Beaver Oregon to examine the effectiveness of Bravo fungicide (Chlorothalonil) in controlling Swiss needle cast (SNC) on Douglas-fir. The selected stand was identified as being severely infected with the disease in 1995. Three 5-acre plots were aerial sprayed with Bravo for five consecutive years (1996-2000). These three sprayed plots also had adjacent unsprayed control plots. The objective of this study is to examine the impact of Bravo on wood properties of the trees. Growth and SNC intensity are being examined in another report.

Methods

In the fall of 2000, when the stand was 20-years-old, growth plots were established in each of the six plots (3 sprayed and 3 unsprayed). Trees were felled and breast-height disks were sampled from approximately 20 trees in each plot for a total of $6 \times 20=120$ disks. Each disk was examined ring-by-ring using X-ray densitometry for the following characteristics: ring width, earlywood width, latewood width and wood density.



Moisture content of the sapwood and heartwood was examined on May 30 and September 10 of 2001 from different samples of trees. Twenty dominant trees in each of the six plots (three sprayed and three controls) were cored with a 5 mm increment borer from bark to pith and separated into sapwood and heartwood. The data from the May sample date have been thoroughly examined, only preliminary results are available for the September sampling date for this report. Specific gravity and moisture content were determined by obtaining green weight (fresh

weight), dry weight and core length. By using a wood density conversion factor of 1.53 g/cm≥ it was possible to determine the volume of wood, water and air in the wood components. Calculations were as follows:

Core volume = core length × π × (0.25≤)

Moisture content (dry weight basis) = 100× [(green weight – dry weight) / dry weight]

Wet density = green weight / volume

Dry density = dry weight / volume

% water (by volume) = 100 × [(green weight† – dry weight) / volume]

% wood (by volume) = 100 × [(dry weight / 1.53) / volume]

% air (by volume) = 100 - %water - %wood

Results

Examination of the growth rings from the plots destructively sampled for the growth study showed that the growth of the trees sprayed with Bravo appeared to have recovered to a "normal" growth rate after two years of spraying since ring width had increased and stabilized by the third growing season in the sprayed plots (Table 1). Growth was reduced in both the earlywood and latewood growth rings of the unsprayed trees, with the greatest difference observable in the earlywood. This resulted in a higher proportion of latewood in the unsprayed control plots, which in turn, increased ring density.

There was a statistically significant reduction in moisture content of the sapwood in the unsprayed plots on both sample dates; moisture content in sprayed and unsprayed plots was 110% to 88%, respectively in May and 104% to 84% in September. Because of the decreased moisture content, the wet density of the sapwood was less in the unsprayed treatment (Table 2). The heartwood moisture contents did not differ between the sprayed and unsprayed blocks. Further examination of the May data showed that wood density was slightly greater in the unsprayed plots (significant at p = 0.10). There was also a tendency for more sapwood area in the sprayed blocks (235 vs. 176 cm≤, p=0.1261).

All heartwood was laid down before spraying occurred, and as expected, there were no statistical differences (p=0.05) between the heartwood of the two treatments for the percent of wood, water or air in both the May and September samples. For both May and September there were significant differences in the volume of water and air between the two treatments in the sapwood (Figure 1). Trees sprayed with Bravo had approximately 50% of their sapwood

Table 1. Average ring widths (cm), latewood proportion and ring density determined from growth plot disks. (Bravo plots were sprayed from 1996 to 2000)

Ring year	Ring Width		Earlywood width		Latewood width		Latewood proportion		Ring density	
	Control	Bravo	Control	Bravo	Control	Bravo	Control	Bravo	Control	Bravo
1992	0.605	0.535	0.265	0.219	0.340	0.317	0.565	0.596	0.561	0.596
1993	0.657	0.646	0.336	0.316	0.321	0.330	0.494	0.528	0.501	0.519
1994	0.575	0.589	0.257	0.268	0.318	0.321	0.566	0.562	0.550	0.562
1995	0.472	0.471	0.228	0.208	0.243	0.263	0.525	0.565	0.555	0.582
1996	0.363	0.340	0.135	0.120	0.228	0.220	0.636	0.660	0.615	0.645
1997	0.395	0.465	0.157	0.175	0.238	0.290	0.608	0.624	0.594	0.613
1998	0.398	0.631	0.204	0.385	0.194	0.246	0.497	0.402	0.561	0.514
1999	0.326	0.599	0.154	0.349	0.172	0.249	0.539	0.426	0.579	0.517
2000	0.305	0.654	0.143	0.390	0.162	0.264	0.529	0.409	0.602	0.529

Table 2. Mean and standard errors of moisture content, wood density and volumetric percentage of wood, water and air in the heartwood and sapwood of trees sampled in May 2001.

	Sapwood				Heartwood			
	Unsprayed control		Bravo-sprayed		Unsprayed control		Bravo-sprayed	
	Mean	std err	Mean	std err	Mean	std err	Mean	std err
Moisture content	87.9	0.8	109.6	2.3	36.1	0.5	36.4	0.5
Density (dry)	0.480	0.008	0.458	0.006	0.420	0.005	0.439	0.006
Density (wet)	0.897	0.011	0.954	0.004	0.571	0.006	0.599	0.008
Area	175.5	3.5	238.7	26.0	86.3	7.0	100.5	16.7
% Wood	31.3	0.49	29.9	0.38	27.4	0.34	28.7	0.38
%Water	41.8	0.49	49.6	0.60	15.1	0.19	16.0	0.31
% Air	26.9	0.87	20.4	0.41	57.4	0.45	55.3	0.65

volume filled with water in May, while the unsprayed trees had only 42%. The September percentages were 47% for the sprayed trees and 40% for the unsprayed. This appears to be a true reduction in moisture in the untreated trees since there was a statistically significant increase in percentage of air, but not in the percentage of wood.

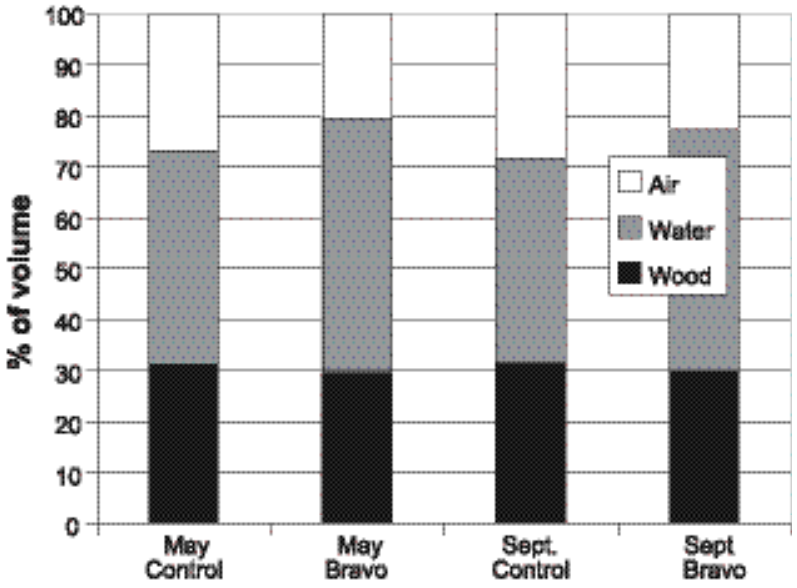


Figure 1. Volume of wood, water and air in the sapwood of plots sprayed with Bravo and unsprayed controls on two sample dates (May and Sept 2001).

Discussion

Compared to the unsprayed trees, the Bravo-sprayed trees showed increased growth rates, lower percentages of latewood, lower ring density and higher moisture contents. It appears that the sapwood of severely SNC-impacted trees have a diminished capacity of water transport relative to healthy trees. While we were not able to distinguish between water being transported in the tracheids and intercellular water (which is not being transported), the reduction we found in moisture content could not be accounted for by intercellular water alone.

It has been shown that air embolisms in the xylem can be refilled by trees, however, the exact mechanism is unknown. Experiments with phloem-girdled stems suggest that photsynthate is required to refill an embolism to recover specific conductivity (Salleo et al. 1996; Zwieniecki and Holbrook 1998) and moisture content (Taylor 1999; Wilson and Gartner in press). Thus this reduction in moisture content could be a function of the tree's overall poor health; there may be an insufficient phosynthate pool to overcome many of the air embolisms that occur on a daily basis.

For a given log size, log weights from the sprayed plots will be greater than those from the unsprayed plots. The sprayed trees have more sapwood, which is of higher wet density than heartwood, and the moisture content of the sapwood is greater in the sprayed trees. The average wet density of the entire pith-to-bark core for the May sample averaged 0.712 in the unsprayed plots and 0.765 in the sprayed plots. Based upon the breast-height disk, a load of logs from the sprayed plots would weight approximately 7.4% more than an equal load (same volume) of unsprayed logs. However, there would be no difference between the dry weight of the loads because the dry densities were identical (0.445 in the unsprayed plots vs. 0.447 in the sprayed plots).

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