O C T O B E R 1 9 9 8

Swiss Needle Cast

SNCC

Cooperative

A N N U A L R E P O R T
1 9 9 8

Forest Research Laboratory
College of Forestry
Oregon State University

Members of the Swiss Needle Cast Cooperative and Their 1998 Contributions

Boise Cascade Corporation	$15,000
Coos County Forestry Department	$5,000
Confederated Tribes of the Grand Ronde	$5,100
Confederated Tribes of the Siletz	$700
Davidson Industries	$5,000
Hampton Resources, Inc.	$10,000
Longview Fibre Co.	$15,000
Menasha Corporation	$15,000
Miami Corporation	$5,000
Oregon Department of Forestry	$15,000
Port Blakely	$5,000
Rayonier	$3,000
Rosboro Lumber Co.	$5,000
Roseburg Forest Products	$5,000
Simpson Timber Co.	$15,000
Starker Forests	$15,000
Swanson Superior Forest Products, Inc.	$5,000
The Timber Company	$15,000
Willamette Industries	$15,000
USDA Forest Service	In kind
USDI Bureau of Land Management	$15,000
OSU Forest Research Laboratory	$30,000

Swiss Needle Cast

SNCC

Cooperative

ANNUAL REPORT

1998

Edited by Greg Filip, SNCC Coordinator

Layout by Gretchen Bracher, FRL Publications

SNCC Income Sources and Expenditures 1998

Income	
Membership Dues	$188,800
Balance from 1997	$93,422
Total Income	$282,222
Expenditures	
Salaries and Wages	$98,407
OPE	$27,694
Supplies and Services	$98,766
Travel	$6,019
Indirect Costs	$20,998
Total Expenditures	$251,884
Balance	**$30,338**

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To: SNCC Members
From: Greg Filip
Date: October 1998
Subject: 1998 Annual Report

This is the second year for SNCC, and I thought that we should have a fancy annual report this year. I think that the members deserve it for all of the support that they have given SNCC this year. It certainly has been a busy year for SNCC. We conducted a successful field trip this April for 75 people near Tillamook. We are actively conducting at least seven projects this year. This year's annual report contains summaries on the progress made on our projects this year. We had two aerial survey projects this year, one in Oregon and one in Washington for the first time, that show a continuing intensification of Swiss needle cast. The final report on the growth impact study has been distributed, and this annual report contains a summary of that work. Progress continues on the basic infection biology research which is summarized in this report. Projects are continuing in needle physiology and tree genetics, and progress reports are contained in this report.

I would like to especially thank our 1998 investigators for their fine efforts in generating new information concerning Swiss needle cast: Alan Kanaskie, Doug Maguire, Katy Kavanagh, Jeff Stone, and Randy Johnson. 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, John Washburn, Greg Johnson, Dale Claussen, Bill Atkinson, Jim Carr, and Alan Kanaskie. We have ten projects planned for 1999; it should be another exciting and productive year.

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Highlights of 1998

This report presents the Swiss Needle Cast Cooperative activities in Swiss needle cast research. Highlights for 1998 include:

- We conducted an "executive field tour" on April 28 in the Tillamook, OR area. There were over 70 participants that viewed Swiss needle cast symptoms in complete sunshine!
- An aerial survey was conducted over 2.2 million acres in Oregon. A total of 173,000 acres of Douglas-fir had obvious symptoms on Swiss needle cast. Survey maps can be obtained from Alan Kanaskie, Oregon Department of Forestry in Salem.
- An aerial survey was conducted over 1.7 million acres in southwest Washington. A total of 44,500 acres had symptoms of Swiss needle cast. Survey maps can be obtained from Dan Omdal, Washington Department of Natural Resources in Olympia.
- Phase I and II of the growth impact study has been completed by Doug Maguire. Results have been written in a separate report and are summarized in this report. Results show a 22% volume growth loss over the entire sampling area with a projected loss of 1,668 MMBF over a 40-year rotation.
- Several new findings concerning fungal and host biology and genetics are presented here in this annual report.

Aerial Survey for Swiss Needle Cast, 1998

Alan Kanaskie and Mike McWilliams

The attached map (Figure 1) shows the approximate size and location of areas of Douglas-fir forest with symptoms of Swiss needle cast detected during an aerial survey conducted in April, 1998. Also included for comparison are aerial survey maps for 1996 and 1997 (figures 2, 3, and 4).

Survey procedures:

Flights were made 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 abnormally yellow to yellow-brown foliage, a symptom of Swiss needle cast. Patches of forest with these symptoms (the patches are referred to as polygons) were drawn onto 1:100,000 scale topographic maps. Each polygon was classified for degree of discoloration as either "L" (light) or "H" (heavy). Polygons classified as "H" for discoloration had very sparse crowns and brownish foliage, while those classified as "L" were predominantly yellow to yellow-brown foliage and slightly

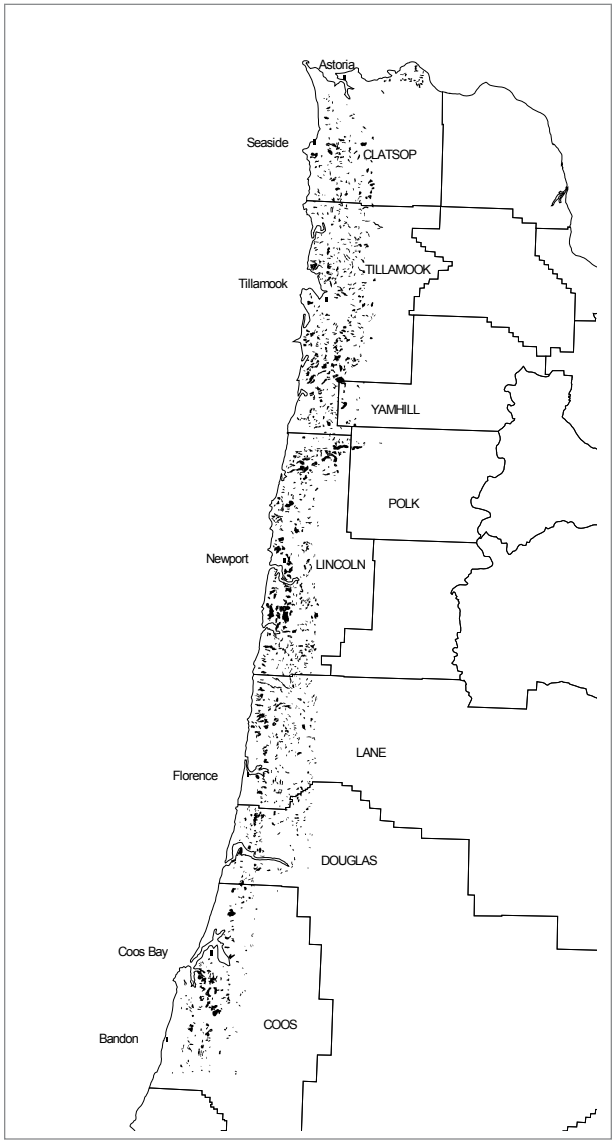

Figure 1. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April, 1998.

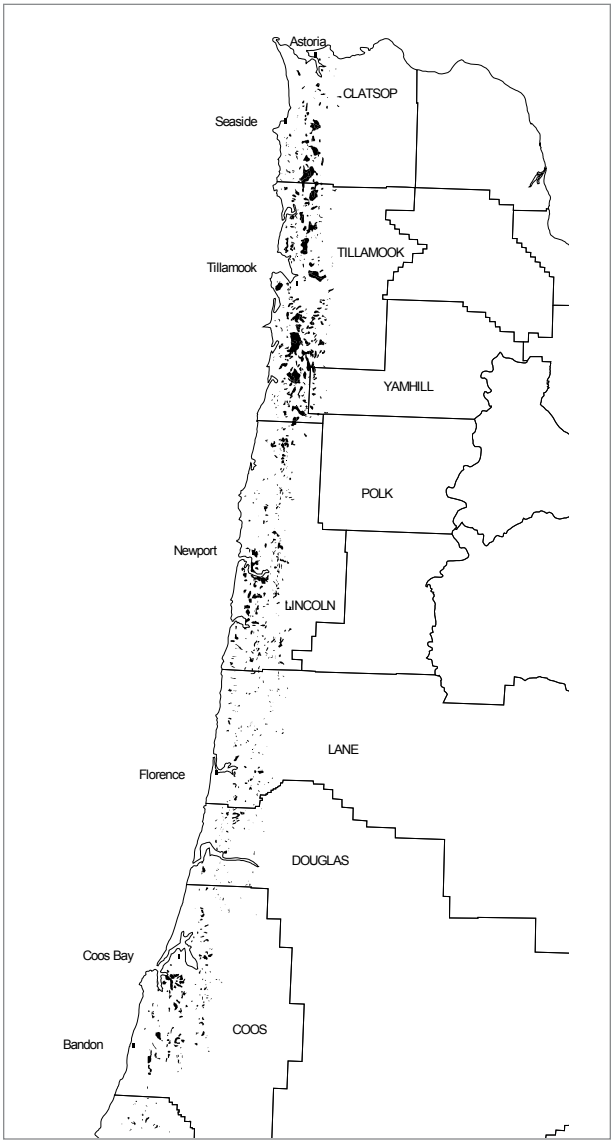

Figure 2. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April, 1996.

more dense crowns than those classified as "heavy". Unlike previous surveys, we did not classify stands by tree age class.

The area north of Newport was surveyed on April 16, 20, and 21. The area south of Newport was surveyed on April 22 and 27. The survey extended from the coast eastward until obvious symptoms were no longer visible, and from the Columbia river south to near Bandon (43 degrees north latitude).

Results of the survey:

The survey covered about 2.2 million acres of forest. A total of 173,000 acres of Douglas-fir forest had obvious symptoms of Swiss needle cast; 135,000 acres north of Florence, and 38,000 acres south of Florence.

The fluctuation in acres mapped between 1996, 1997 and 1998 is partially due to our re-flying the northern part of the survey in May of 1997. After the initial survey was completed in 1997, favorable weather enhanced symptom development and we re-surveyed the northern part of the region (Yamhill and Nehalem quads) in mid-May. This was nearly a month later than it was surveyed in the initial 1997 survey and in either 1996 or 1998. If we ignore the re-survey and use only the initial 1997 survey results, the acres mapped in 1997 are reduced from 393,000 acres to 145,000 acres (table 1).

Because symptoms develop rapidly during April and May, later surveys will detect more areas than those conducted earlier. However, the seasonally unstable weather requires that we begin the survey early enough to ensure completion. It is likely that the 1997 survey overestimated the extent of symptoms that could reasonably be detected during an average year, under average conditions. However, it also indicates the conservative nature of

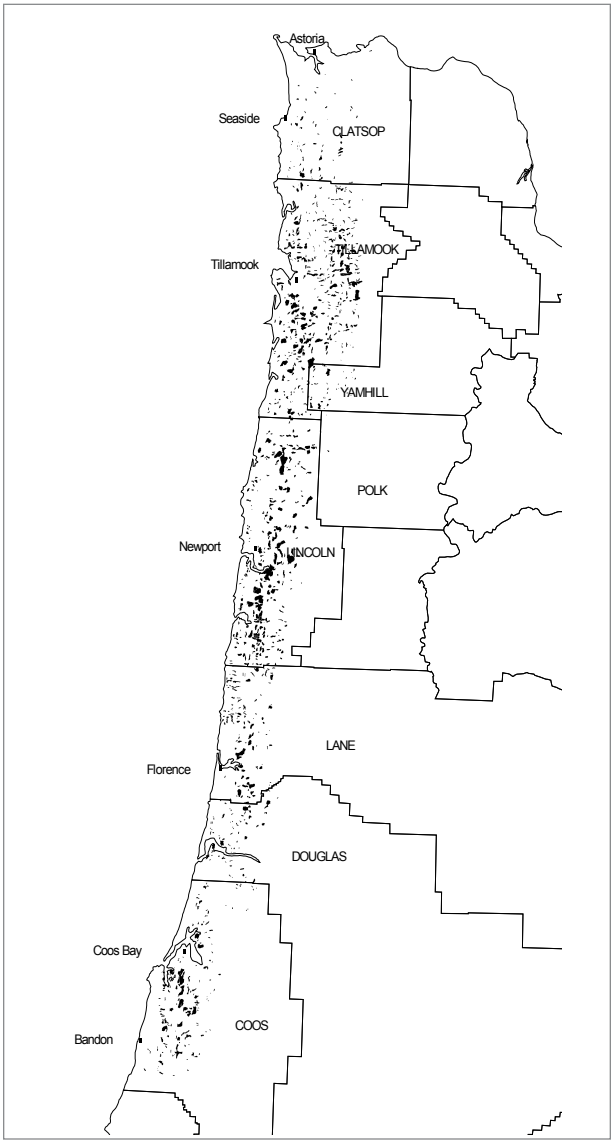

*Figure 3. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May, 1997 (does **not** include re-fly of Nehalem and Yamhill quadrangles).*

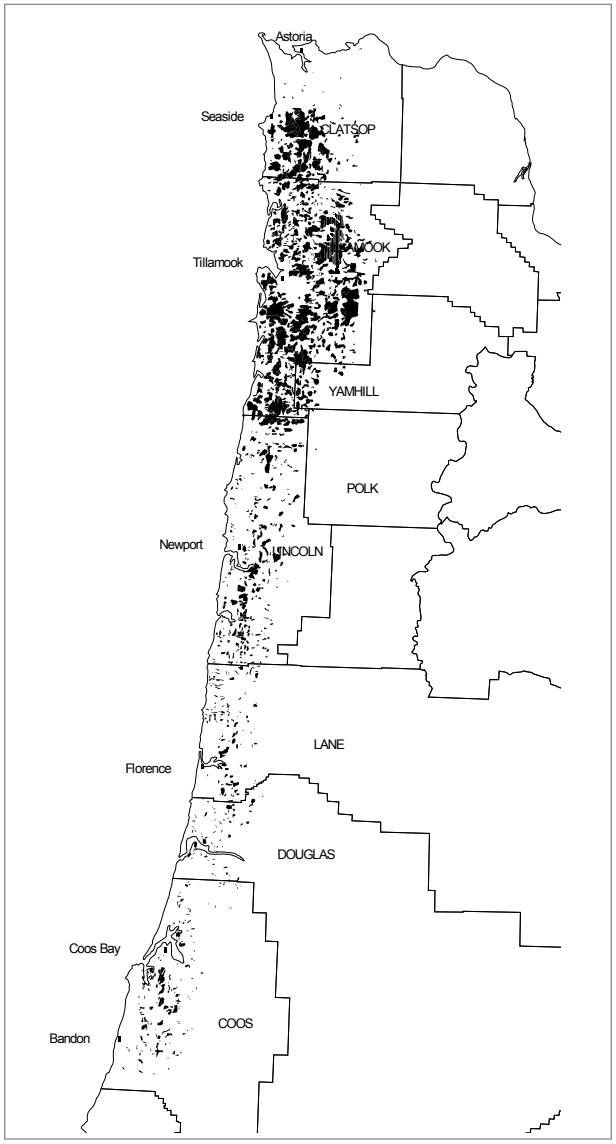

Figure 4. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May, 1997 (includes re-fly of Nehalem and Yamhill quadrangles).

Table 1. Area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in 1996, 1997, and 1998. In 1997 the Nehalem and Yamhill quadrangles were surveyed initially in April and re-surveyed in May.

Region	1996	1997 (with re-survey)	1997 (initial survey only)	1998
		acres		
North of Florence	106,000	363,000	115,000	135,000
South of Florence	24,000	30,000	30,000	38,000
TOTAL	130,000	393,000	145,000	173,000

the survey, and the enormous influence of weather and time on the dynamic process of symptom development.

After three years of surveys, we have little doubt that Swiss needle cast symptoms continue to intensify. It is also clear that even though the disease occurs throughout the Coast Range, most areas with symptoms that can be detected from the air are within about 18 miles of the coast.

These estimates still must be considered conservative because they represent only those areas with obvious symptoms. The aerial survey does not depict the total extent and distribution of the disease, but only those areas where disease symptoms have developed enough to be visible from the air. Ground surveys indicated that Swiss needle cast occurred in all Douglas-fir stands throughout the survey area, but in many places symptoms were not developed enough to enable aerial detection. Factors other than the presence of the pathogen strongly affect disease development, and these factors remain poorly understood. The shape and distribution of polygons on the survey maps suggest that symptoms are most obvious on southerly aspects and on exposed ridge tops, indicating a strong environmental interaction.

Most polygons that were ground checked confirmed that Swiss needle cast was present, and that symptoms observed were consistent with this disease. One exception to this was an area of discoloration near Laurel Mountain (T7S, R7W). Ground checks in this area showed yellow Douglas fir with normal needle retention and very little evidence of the Swiss needle cast fungus, suggesting site or other environmental factors as the cause of discoloration. This area was not included on the attached map.

Timing of aerial surveys:

As was the case in previous years, foliage discoloration intensifies through April, with the most pronounced discoloration occurring about two weeks before and one week after bud-break. Ideally, aerial surveys would be conducted during this time, which usually is the first two weeks in May. However, typically unstable spring weather requires starting in April to ensure completion of the survey. In the interest in consistency from year to year, we do not recommend re-flying areas when the chance weather provides for good symptoms and aerial detection late in the survey period. Rather, we suggest beginning the survey as weather permits on or after April 15.

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. Jack Prukop (ODF) piloted the plane. Mike McWilliams (ODF), Keith Sprengel (US Forest Service), and LeRoy Kline (ODF) were the aerial observers.

We appreciate any information regarding the accuracy or usefulness of the maps. If you have a chance to look at some of the mapped areas on the ground, please let us know what you observe. Please call Alan Kanaskie (503-945-7397) or Mike McWilliams (503-945-7395) if you have questions or comments.

Growth Impact Study: Progress report from Phases I and II

Doug Maguire, Alan Kanaskie, Bill Voelker, Randy Johnson, and Greg Johnson

Damage from SNC has been observed in the past on Douglas-fir grown outside of its natural range, first in Europe in the early part of the century, and more recently in both Christmas tree plantations throughout North America and timber stands in New Zealand. Although some European and New Zealand research was motivated by concern about growth losses, relatively little work has been accomplished to date on estimation of growth impact especially within the natural range of Douglas-fir and across a gradient in infection severity. Height growth of SNC-affected Douglas-fir in the Pacific Northwest has been estimated at 50-70% of normal height growth, corresponding to as much as a 30% volume growth loss (USDA-FS 1983; as cited in Russell et al. 1986). In New Zealand, basal area growth of stands experiencing Douglas-fir decline was as low as 50% of the basal area growth of stands exhibiting no decline (Beekhuis 1978). An analysis of Douglas-fir plots planted in Kaingaroa Forest indicated that stands planted since appearance of SNC had markedly lower (by 26%) basal area growth for a given top height than did stands growing prior to emergence of SNC (Manley 1985). The Growth Impact Study described below was initiated by the Swiss Needle Cast Cooperative to address three specific objectives: 1) to identify tree and foliage attributes that may serve as indices of SNC severity and corresponding growth losses; 2) to estimate the current losses of stem volume growth across the range in SNC severity; and 3) to gain some insight into past growth trends of plantations that are currently exhibiting varying levels of SNC.

Methodology

Target population

The target population for the growth impact study was defined as Douglas-fir plantations between 10 and 30 years of total age, located within 29 km (18 miles) of the coast, north of Newport and south of Astoria. A list of plantations meeting these criteria was assembled, and plantations were selected from this list with probability proportional to size (area). The target population included 4504 plantations covering 75,929 ha (187,545 ac). The sample included 79 plantations covering 2783 ha (6873 ac). Each plantation was assessed for SNC intensity, and 70 of the 79 plantations were then measured intensively to establish the link between SNC rating and growth loss.

Field work

In March and April of 1997, a five-point transect was established from a random location along the access road for each plantation. Sample points were separted by 15 m (50 ft), resulting in a 75-m (150-ft) transect. Color attributes and needle retention were estimated on the two dominant or codominant Douglas-fir trees nearest to each sample point.

The third sample point on 70 of the sample transects was flagged, marked with a PVC stake, and subsequently used as the center of a 0.02-ha (0.05-ac plot; radius=26.3 ft or 8.01 m) on which all trees were measured for dbh (diameter outside bark at 1.37 m). The four Douglas-fir trees with the largest dbh and two with the smallest dbh were felled, as were the two Douglas-fir closest to the mid-range of the diameter distribution. Each felled tree was measured for total height, height to crown base, and cumulative height growth (height of each bud scale scar, at or just above each annual branch whorl). A sample branch was collected from the fourth largest Douglas-fir tree on each plot. Breast height stem disks were brought to the lab and measured for annual radial growth and sapwood cross-sectional area. Sample branches were analyzed for mass, area, and pseudothecia count by age class.

During the summer of 1997, a subset of plots was revisited and all live Douglas-fir trees were measured for height to crown base and total height (ignoring the 1997 leader growth). Radial growth and sapwood area were estimated on increment cores removed from each standing Douglas-fir tree.

Analysis

Patterns in past basal area and height growth were reconstructed from the annual height increments and radial growth measurements. In addition to the SNC ratings of standing trees on the sample transects, tree and sample branch measurements from the growth impact plots allowed computation of many alternative indices of SNC intensity (Table 1).

Initial height and height growth for each annual growth period were reconstructed for all felled Douglas-fir trees. Similarly, initial diameter outside bark (dob) and inside-bark basal area growth for each annual growth period were estimated on all Douglas-fir trees from which a breast height disk or increment core was removed. Various stand and tree variables were then estimated for each annual

growth period represented in the height growth and diameter growth records.

Height growth and basal area growth, the two major components of volume growth, were analyzed separately since it was evident early in the study that the onset of growth reductions in these two components were asynchronous and that their relative magnitudes were different. An analysis of top height growth of the plots was implemented by computing potential predictor variables and subjecting the data to an all-subsets regression analysis, with the logarithm of annual height growth as the response variable. In the final models, the "effect" of SNC was assessed by fitting the growth model on an annual basis, computing the % of expected growth across the range in the selected SNC index, and graphing this % of expected growth from 1978 through 1996. In this approach, growth of the plantations with the least severe SNC ratings served as the expected growth rate, after correcting for the effects of other covariates in the regression model (that is, holding all other variables such as site quality and initial size constant). Basal area growth was assessed in the same way.

RESULTS

The 70 growth impact plots ranged between 8 and 30 years of total age, and all were Douglas-fir plantations with varying amounts of naturally regenerated western hemlock. Sampled plantations covered a range in total conifer stand density (Table 2), individual tree condition (Table 3), and SNC status (Table 4).

The final model for top height growth illustrated the apparent relationship between mean foliage retention and trends in **top height growth**:

$$\ln(\Delta th) = a_0 + a_1 \bullet X_1 + a_2 \bullet \ln(retx) \qquad R^2 = 0.14 - 0.63 \qquad [1]$$

where $\ln(\bullet)$ = natural logarithm

Δth = annual top height growth (m/yr)

retx = average years of foliage retention

X_1 = Bruce's (1981) predicted top height growth (m)

$= 0.3048 \bullet \ln(hite \bullet b_2 \bullet b_3 \bullet (piage+13.5-(SI_{90}/0.3048)/20)^{(b3-1)})$

with

piage = average initial breast height age at the start of the growth period

$hite = SI_{90} \bullet \exp(b2 \bullet ((piage+13.25-(SI_{90}/0.3048)/20)^{b3} - (63.25-(SI_{90}/0.3048)/20)^{b3}))$

$b_3 = -0.447762 - 0.894427 \bullet ((SI_{90}/0.3048)/100) + 0.793548 \bullet ((SI_{90}/0.3048)/100)^2 - 0.171666 \bullet ((SI_{90}/0.3048)/100)^3$

$b_2 = \ln(4.5/(SI_{90}/0.3048))/((13.25-(SI_{90}/0.3048)/20)^{b3} - (63.25-(SI_{90}/0.3048)/20)^{b3})$

SI_{90} = Bruce's (1981) site index computed from plot data backdated to 1990 (m at 50 years)

and

a_0, a_1,and a_2 are regression parameters estimated from the data for each year of backdated height growth

The top height growth losses in plantations with the most severe SNC infection intensities were as high as 25%, but averaged about 10% (Fig. 1). Significant top height growth reductions appeared to have started between 1990 and 1992.

The final model for tree **basal area growth** illustrated the relationship between mean foliage retention and diameter growth:

$$\ln(\Delta ba) = c_0 + c_1 \bullet \ln(idob) + c_2 \bullet idob^2 + f_3 \bullet sl + c_4 \bullet BAL + c_5 \bullet \ln(SDI) + c_6 \bullet \ln(retx) \qquad R^2 = 0.62 - 0.91 \qquad [2]$$

where $\ln(\bullet)$ = natural logarithm

Δba = annual basal area growth of individual tree (cm^2/yr)

idob = tree DBH at start of growth period (cm)

BAL = basal area in trees with larger dbh than the subject tree (m^2/ha)

sl = slope %

SDI = stand density index = $tph \bullet (Dq/25.4)^{1.6}$

retx = average years of foliage retention

and

c_0, c_1, c_2, c_3, c_4, c_5, and c_6 are regression parameters estimated from the data for each year of backdated basal area growth

The basal area growth losses of trees in plantations with the most severe SNC infection intensities were as high as 35%, but averaged about 15% (Fig. 2). Significant basal area growth reductions appeared to have started between 1988 and 1990.

Volume growth is controlled largely by two major components, basal area growth and height growth. Taking the associated values of 90% and 85% for the average percentages of expected height and basal area growth respectively (Figs. 1 and 2),

Table 1. Potential indices of SNC severity.

Attribute	Definition and units
yx	Yellowness index (6=most yellow, 0=not yellow)
retx	Mean foliage retention (yrs)
len1	Ave. length of 1-yr-old needles (mm)
sla1	Specific leaf area of 1-yr-old needles (g/cm^2)
sla	Specific leaf area of all needles (g/cm^2)
pret1	Percent foliage retention of 1-yr-old needles
pret2	Percent foliage retention of 2-yr-old needles
pret	Percent foliage retention of 1-3-yr-old needles
fm1	Foliage mass on 1-yr-old shoots (g)
fm2	Foliage mass on 2-yr-old shoots (g)
fm3	Foliage mass on 3-yr-old shoots (g)
tfm	Foliage mass on 1-5-yr-old shoots (g)
pfm1	Percent foliage mass in 1-yr-old needles
pfm2	Percent foliage mass in 2-yr-old needles
p1	Pseudothecia count on 1-yr-old needles (0-100)
p2	Pseudothecia count on 2-yr-old needles (0-100)
p3	Pseudothecia count on 3-yr-old needles (0-100)
lmba	Ratio of foliage mass to branch basal area (g/mm^2)
pclsa	Plot average ratio of live crown length to sapwood area at crown base (cm/cm^2)

Table 2. Means and ranges of sample stand attributes.

Attribute	Min	Mean	(sd)	Max
Total age (yrs)	8	17	5.4	30
Breast height age (yrs)	4	13	4.9	29
Site index (1990; ft at 50 yrs)	23.4	39.3	4.6	47.2
Trees per ha				
All species	247	1627	2223	17142
Douglas-fir	148	688	347	927
Hardwood	0	349	839	5088
Quadratic mean dbh (cm)				
All species	3.63	15.27	6.86	33.89
Douglas-fir	5.52	17.81	6.63	33.56
Hardwood	0	4.28	5.98	35.80
Basal area (m^2/ha)				
All species	2.84	19.25	10.96	54.97
Douglas-fir	2.03	15.77	9.02	37.24
Hardwood	0	1.09	2.63	15.31
Stand density index (25.4-cm trees per ha)				
All species	91	468	255	1430
Conifer	91	427	235	1430
Relative density ($m^2/ha/cm^{1/2}$)				
All species	1.04	4.99	2.74	15.48
Conifer	1.04	4.52	2.50	15.48

Table 3.Means and ranges of Douglas-fir sample tree attributes.

Attribute	Min	Mean	(sd)	Max
DBH (cm)	0.4	14.8	7.7	40.3
Heights (m)	2.09	11.28	4.83	31.08
Crown length (m)	0.16	8.58	3.62	20.34
Sapwood area, crown base (cm^2)	0.4	136.9	107.3	836.4
Crown length/ sapwood area (cm/cm)	1.3	10.2	9.7	100.6
Breast height age (yrs)	1.0	11.5	5.1	30.0
Basal area growth (cm^2)	0.3	22.0	16.7	112.5
Top height growth (m)	0.00	0.91	0.19	1.64
Basal area in larger trees (m^2/ha)				
All species	0.00	11.06	9.41	54.85
Douglas-fir	0.00	9.13	7.83	35.87
Hardwood	0.00	0.46	1.42	7.74

Table 4.Means and ranges of SNC severity indices (see Table 1 for variable definitions).

Attribute	Min	Mean	(sd)	Max
yx	0.00	1.29	0.65	2.90
retx	1.09	2.20	0.52	3.65
len1	13.9	23.3	3.5	30.9
sla1	56.53	69.44	8.91	96.71
pret1	0	81	25	100
pret2	0	49	35	100
pret	0	51	24	96
fm1	7.7	91.7	52.3	255.7
fm2	0.5	48.9	31.7	143.2
fm3	0.0	20.1	19.6	90.4
tfm	25.4	162.2	91.2	439.2
pfm1	17	59	17	99
pfm2	1	29	10	46
p1	0	4	5	29
p2	0	35	17	80
p3	0	42	18	80
lmba	10.6	41.7	17.0	108.7
pclsa	3.4	6.4	1.9	12.2

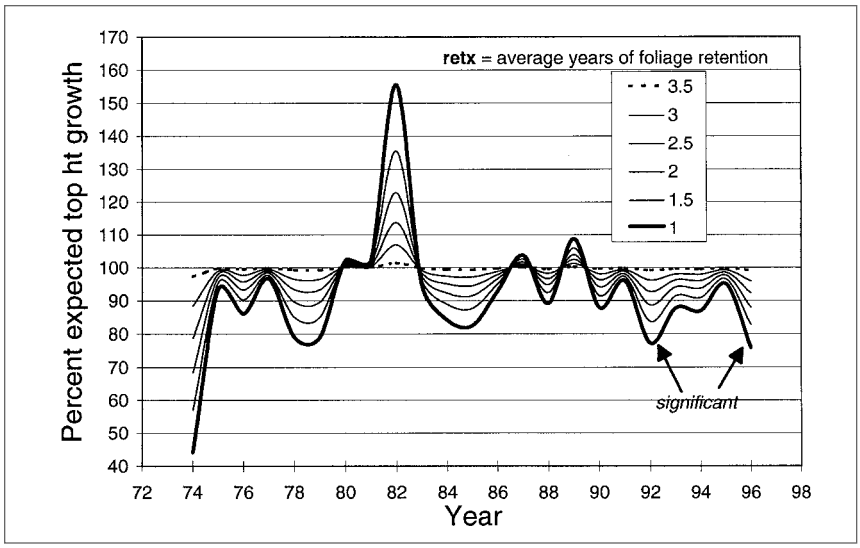

Figure 1. Trend in relative top height growth across the range in SNC severity indicated by mean foliage retention (retx).

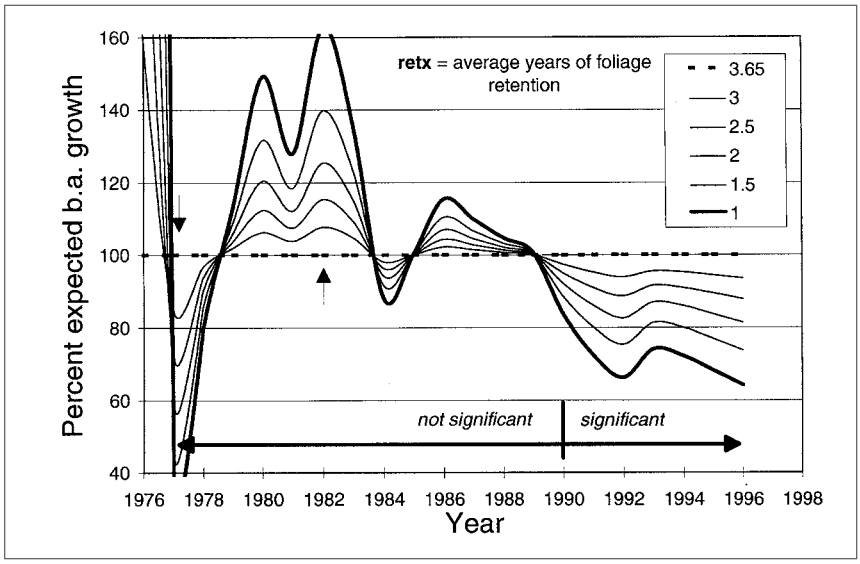

Figure 2. Trend in relative basal area growth across the range in SNC severity indicated by mean foliage retention (retx). Vertical arrows indicate individual years in which foliage retention (retx) had a significantly negative or positive correlation with basal area growth.

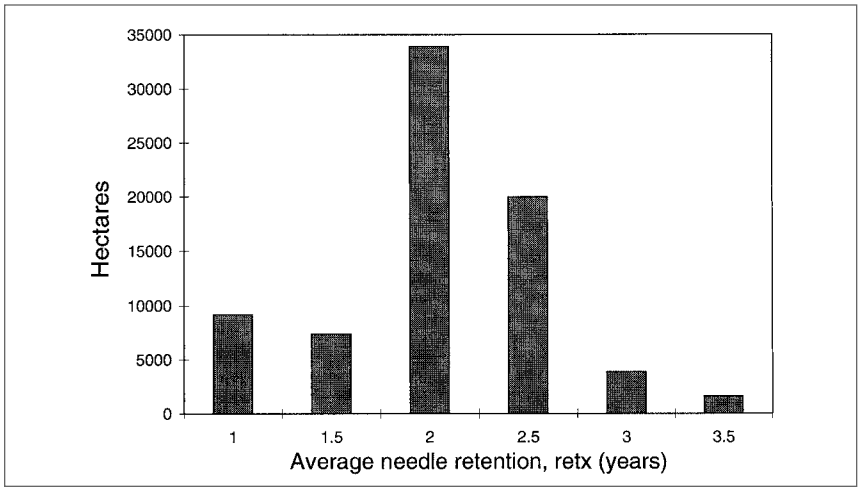

Figure 3. Estimated distribution of population area by foliage retention class.

the corresponding volume growth was approximately 77% of expected growth (0.90 • 0.85 = 0.77). This estimate suggested an average 23% growth loss in 1996 for the entire population. In slightly older plantations growing at a rate of 14 m^3/ha/yr (1,000 board-feet /acre/year), the implied growth loss would be about 3.2 m^3/ha/yr (230 board feet/acre/year) for 1996 alone, or about 244,000 m^3 (43 million board feet) per year for the entire population. At the low extreme, plantations retaining only a single age class of needles were estimated to have experienced a 51% volume growth loss in 1996.

The distribution of acreage across foliage retention classes (Fig. 3) indicated that approximately 2/3 of the target population had an average needle retention of 2 years or less and approximately 1/2 of the population experienced a growth loss of 30% or greater (Figs. 3 and 4). Hence, approximately 37,000 ha (92,000 ac) of Douglas-fir plantation experienced a volume growth loss of 30% or greater in 1996, and approximately 11,000 ha (28,000 ac) experienced a volume growth loss of 40% or greater.

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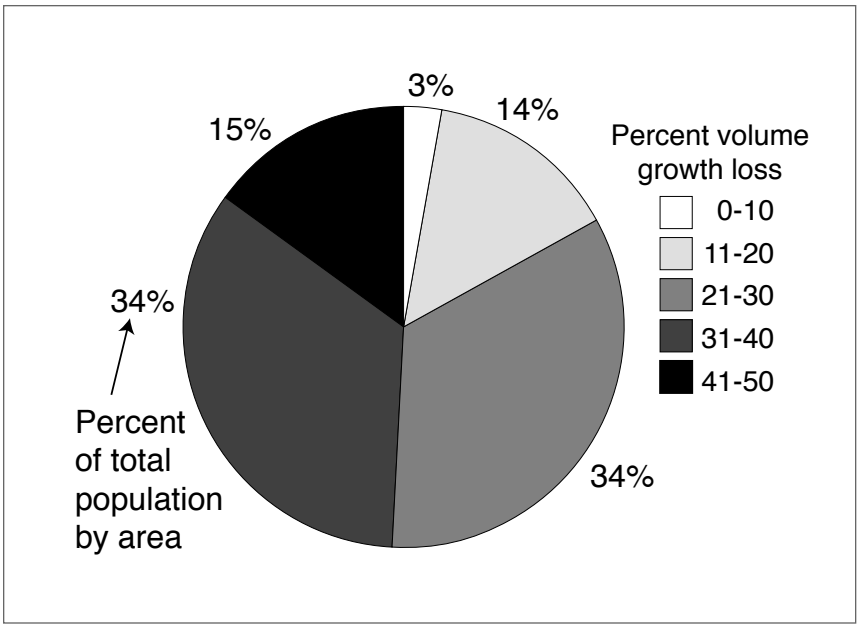

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Stomata Response to Swiss Needle Cast in Douglas fir; Understanding the Interaction Between Symptoms and Site Conditions.

Progress Report - Fall 1998

Dan Manter, Kathleen Kavanagh, Jeff Stone, Pablo Rosso, and Greg Filip.

Objective

Determine the impact of Swiss needle cast (SNC) caused by *Phaeocryptopus gaeumannii* (PG) on Douglas-fir gas exchange and stomatal function, and potential interactions with environmental conditions.

Specific goals

1. Develop and compare methods for quantifying PG, i.e., biomass index (ergosterol), reproductive structures (pseudothecia counts), and needle colonization (culture methods).
2. Investigate the limitations that SNC may impose on Douglas-fir photosynthesis.
3. Measure net assimilation and stomatal conductance on foliage with varying levels of SNC.
4. Determine potential interactions between PG infection and environment.

Rationale

Typically, PG infection is measured by visible symptoms (needle retention and chlorosis) and/or pseudothecia production. How such measures correlate with one another, other aspects of fungal growth, or their relation to host physiology is not known. Depending on the mechanism by which SNC is influencing host physiology, it may be preferable to quantify SNC by other methods. For example, a measure of fungal biomass may correlate better with fungal enzyme production and any fungal enzymatic control of host physiology, whereas pseudothecia production may best correlate with host function as determined by stomatal blocking and reduced CO_2 uptake.

Foliar fungi extract the nutrients necessary for their survival from the plant tissues that they invade, consequently reducing host growth and vigor. In addition to direct absorption of nutrients, fungi may also reduce host photosynthate production by enzymatic and/or non-enzymatic means. Enzymatic means include changes in host cellular processes and enzymes (e.g., rubisco, Walters and Ayres, 1984) or fungal enzymes that abnormally regulate host physiology (e.g., invertase, Tang et al. 1996). Non-enzymatic means include the loss of host tissue and physical blocking of stomata. Understanding the relative contribution of enzymatic and non-enzymatic limitations of PG infection on host function is a necessary step in determining the mechanism(s) by which PG causes reductions in growth and productivity.

Of the methods above, preliminary data suggest that PG mainly impacts Douglas-fir foliage through non-enzymatic processes. First, PG infection results in needle abscission which reduces the total amount of foliage available for photosynthate production. Second, PG infection reduces the productivity of the remaining infected foliage. As PG matures it produces reproductive structures, pseudothecia, which emerge from stomata, thus limiting their ability to uptake CO_2 , reducing photosynthesis and growth of infected foliage. What we currently do not fully understand is the relationship between PG infection levels and resultant reductions in host physiology.

The impact of SNC on host physiology may also be determined by environmental conditions. Stomata open and close in response to environmental conditions such as light, temperature, humidity and water levels, in such a manner as to maximize CO_2 uptake and minimize water loss. Under favorable environmental conditions, infected foliage may be able to compensate for PG infection by leaving their functioning stomata open longer during the day, thereby allowing transpiration and photosynthesis to continue. Even though there is less water movement per hour, the stomata remain open for more hours during the day. However, once stomata become limited by environmental conditions, this compensation can no longer occur and symptom progression is accelerated. Preliminary reports of greater SNC symptom development on south slopes that tend to have higher temperatures and less soil water than north slopes suggest that such an interaction may occur. We hypothesize that such an interaction between PG infection and environmental conditions exists and may help to explain differences in symptom development.

Project 1: Assessment of Fungal Infection.

Re: Goal 1

Methods

PG-infected foliage from 2 lower canopy branches (1 north and 1 south) per twelve trees on two heavily diseased sites

(North Fork [NF] and Juno Hill [JH]) was collected and analyzed for fungal infection based on the following measures.

Pseudothecia counts: 300 stomata from 5 needles per age class were assessed for emergent pseudothecia. Values reported are the percent of stomata with pseudothecia (i.e., PSEUDO).

Ergosterol: 20 needles from each age class (ca. 1265 mg) were analyzed for the presence of ergosterol. Ergosterol is a cell membrane sterol found in most fungi, but not plant tissue, which may serve as an index of total fungal biomass present (e.g., Gessner et al. 1991). Analysis of ergosterol was estimated by HPLC (high performance liquid chromatography) with a LiChrosphere RP-18 column heated to 30°C and 100% methanol as the solvent system. Elution of ergosterol occurred at 10.5 min and quantification of the eluent was assessed by comparison to known concentrations of pure ergosterol. Values reported are expressed as g ergosterol / g dry weight of needles (i.e., ERG).

Surface sterilizations: 5 needles per age class were surface sterilized to remove epiphytic fungi, cut into 2 mm sections and plated on 2% malt extract agar. All fungi that emerged from cut ends were identified and used to calculate the percent colonization of fungi. Presented here are the percent infection of PG (i.e., PHAEO = # segments with PG / # segments * 100) and the percent infection of all fungi (i.e., FUNGI = # segments with any fungi / # segments * 100).

Results

All measures of fungal infection were significantly correlated with one another (Table 1) suggesting that on heavily infected sites relative differences in PG infection may be determined by either pseudothecia counts, ergosterol presence, or culture methods. However, for more detailed studies it may be better to employ one or more measures of PG infection. For example, as determined by ergosterol and culture methods, PG colonization reaches it maximum in 96 JH needles but pseudothecia production does not maximize until the following year (Table 2).

The use of ergosterol content seems to be an applicable index of PG infection for two reasons. First, ergosterol content correlates well with pseudothecia counts and culture method determinations of PG infection (Table 1). Second, once PG reached its maximum levels of infection (e.g., PHAEO: 96 JH needles, Table 2) so did ergosterol content (e.g., ERG: 96 JH needles, Table 2) even though infection by other endophytic fungi continued to increase.

Table 1. Pearson Correlation Coefficients

	Pseudo	Erg	Phaeo	Fungi
Pseudo	1.000	-	-	-
Erg	0.762	1.000	-	-
Phaeo	0.681	0.769	1.000	-
Fungi	0.766	0.818	0.849	1.000

All correlations are significant at $p<0.05$.

Data is from July 1997 samples at North Fork and Juno Hill sites.

Pseudo=percent of stomata with visible *Phaeocryptopus gaeumannii* pseudothecia.

Erg=ergosterol, ug/g DW needles (not species specific, represents *P. gaeumannii* and any other foliar fungi). Phaeo=percent of foliage infected with *Phaeocryptopus gaeumannii* as determined by culture methods. Fungi=percent of foliage infected with any fungi as determined by culture methods.

Table 2. Quantification of Fungi

	July 1997[1]					
	JH[2]			NF		
Measure[3]	97[4]	96	95	97	96	95
Pseudo	0.2^{b}	29.9^{d}	52.5^{f}	0.0^{a}	10.1^{c}	41.7^{e}
Erg	1.9^{a}	16.3^{c}	15.5^{bc}	2.1^{a}	11.1^{b}	16.6^{c}
Phaeo	0.7^{a}	43.6^{c}	44.9^{c}	2.1^{a}	23.4^{b}	47.5^{c}
Fungi	2.5^{a}	71.3^{c}	90.5^{c}	4.0^{a}	47.5^{b}	83.3^{c}

[1]Sample date; each mean represents 2 branches from twelve trees at each site. Means with different letters are significantly different at $p<0.05$.

[2]Site: JH=Juno Hill, low elevation; NF=North Fork, low elevation.

[3]Fungal measurement: Pseudo = percent of stomata with visible *Phaeocryptopus gaeumannii* pseudothecia; Erg = ergosterol, ug/g DW needles (not species specific, represents *P. gaeumannii* and any other foliar fungi); Phaeo = percent of foliage infected with *Phaeocryptopus gaeumannii* as determined by culture methods; Fungi = percent of foliage infected with any fungi as determined by culture methods.

[4] Foliage age class (i.e., year of initiation).

Differences in SNC symptoms agree with the presence of PG as determined by this study. For example, the JH site typically retains two complements of needles and the NF site carries three (JH 95 needles and 96 NF needles were abscised immediately following the July sample date), and according to these measures of infection JH has significantly higher levels of PG infection versus the NF site. Finally, based on this sample it appears that once needles reach about 50% stomata occlusion, needles will be abscised.

A second study incorporating low and moderately infected sites is currently being conducted. Analysis of surface sterilization studies will also be used to examine fungal communities and their association with SNC.

Project 2: Photosynthetic Limitations of SNC Infected Foliage.

Re: Goal 2

Methods

Two year-old Douglas-fir seedlings were inoculated with macerated mycelium of PG. Following inoculation net assimilation versus internal CO_2 (A/Ci) curves were conducted on a monthly basis to determine SNC imposed limitations on photosynthesis. On each sample date one inoculated and one control seedling was measured. Using a Li-Cor 6200, foliage was

placed into a chamber with constant humidity and temperature (±1%) and net assimilation was measured over a range of CO_2 concentrations. Analysis of the A/Ci curve has been conducted to determine maximum photosynthesis (Ps_{max}), CO_2 compensation point (Γ), stomatal limitations to photosynthesis (L_g) and mesophyll limitations (L_*) to photosynthesis by the differential methods outlined in Jones (1985).

Results

At present, the controls and inoculated seedlings differ only in their CO_2 compensation point (Table 3). Most likely it is PG respiration (fungal presence detected by an ergosterol content ca. 6 ug / g dry weight needles) and the release of CO_2 that is responsible for this change in compensation. Of interest is the lack of differences in maximum net photosynthesis and photosynthetic limitations following fungal colonization (Table 3). This preliminary data suggest that fungal colonization alone is not the cause of a decreased photosynthetic capacity; rather, it is not until the emergence of pseudothecia that photosynthetic rates are impacted. Studies will be continued until pseudothecia are produced to determine their effect on photosynthetic limitations. In addition, a second set of seedlings have been inoculated and will be included in future samples.

Table 3. Photosynthetic Limitations

	Control	Infected
Ps_{max}	23.2[a]	21.2[a]
Γ	50.9[a]	106.[ab]
L_g	0.3[a]	0.3[a]
L_*	0.7[a]	0.7[a]

[1]Ps_{max} is the estimated maximum photosyntesis.
Γ is the CO_2 compensation point.
L_g is stomatal limitations to photosynthesis.
L_* is the mesophyll limitations to photosynthesis.
Means with different letters are significantly different at $p<0.05$.

Project 3: Field Measurements of Diurnal Gas Exchange

Re: Goal 3

Methods

Three sites with varying levels of PG infection were chosen for gas exchange measurements. The most heavily infected site is located on the Siuslaw NF in Beaver, OR (i.e., Beaver), the medium infection site is located on the Siuslaw NF in Hebo, OR (i.e., Hebo), and the low infection site is located in the MacDonald Forest near Corvallis, OR (i.e., Mac). At each site, 12 trees from a north and south facing slope were selected, scored for visible symptoms of PG infection (Table 4), and tagged for future gas exchange measurements. Due to the uniform presence of PG on these sites, 6 of the 12 trees were tagged as "controls" and sprayed with Bravo 720 (rate = 66 ml / gal., applied until run-off). Fungicide applications were conducted in May 1998 prior to bud break, at bud break (90% trees had broken bud) and 1 month following bud break. Starting in June 1998, diurnal analysis of gas exchange and water potential were measured. Diurnal measurements included pre-dawn water potentials, bi-hourly gas exchange (Li-6200), and leaf water potential 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 presence (pseudothecia counts and ergosterol content).

Results

Figure 1 shows a typical diurnal gas exchange curve from the heavily infected site at Beaver. As the needles age and PG pseudothecia production increases, there is a corresponding decline in stomatal conductance (Fig 1B) and net assimilation (Fig 1A). Such a decline is not detected in the Mac Forest diurnal curves (Figure 2A & 2B) where pseudothecia production does not increase above 3% (Table 5).

This work is currently in progress and will continue for the next two years as our sprayed controls produce new healthy foliage. Continuation of this study will help us understand Douglas-fir's ability to recover from SNC, and a more detailed analysis of the interaction between PG-infection levels and growth and productivity.

Table 4. Swiss Needle Cast Visible Symptoms - sampled on 980505

	97[1]		96		95		94		93	
Site, Slope[2]	NR[3]	Color	NR	Color	NR	Color	NR	Color	NR	Color
Beaver, S	89	1.75	32	1.04	0	-	0	-	0	-
Beaver, N	96	1.06	70	1.38	1	0.10	0	-	0	-
Hebo, S	89	0.65	64	0.95	20	0.29	2	0	0	-
Hebo, N	100	0.13	95	0.31	38	0.40	5	0.2	0	-
Mac Forest, S	100	0.02	100	0.02	58	0.06	29	0.13	3	0.02
Mac Forest, N	100	0.02	100	0.06	52	0.17	25	0.21	3	0.02

[1]Age class, year of initiation.
[2]Site name; Slope, N=north, S=south.
[3]Values are means of 4 branches from 12 trees per site, slope combination. Branches were chosen from the north and south sides of the tree from each of the top and bottom half of the crown. NR=mean percent needle retention (original values were assessed as either 0, 25, 50, 75 or 100%). Color=mean color index (original values were assessed as either 0, 1, 2, or 3, with 0 = healthy, no chlorosis)

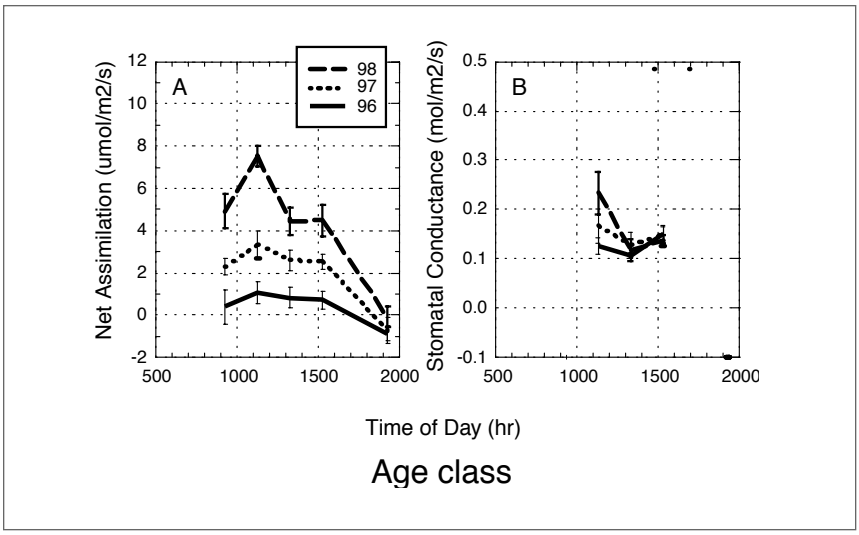

Figure 1. Typical diurnal gas exchange curves from a heavily infected site at Beaver, OR.

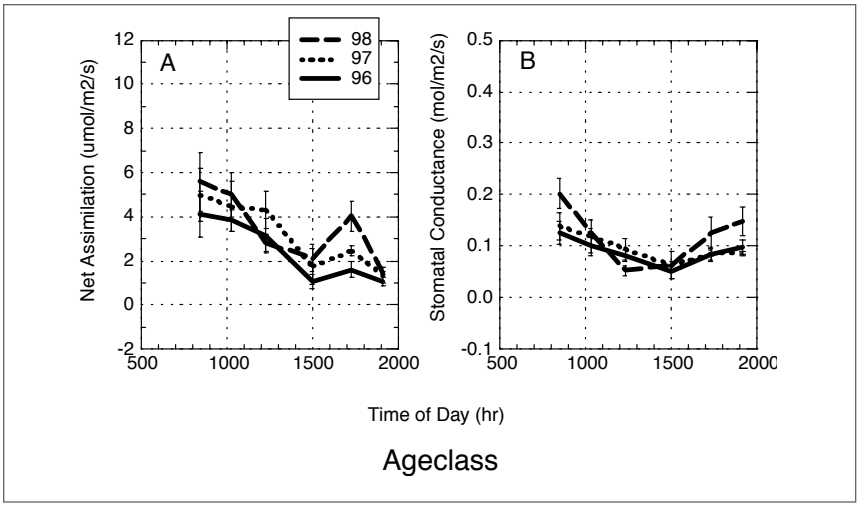

Figure 2. Typical gas exchange curves from a lightly infected site at Corvallis, OR.

Table5. Quantification of Fungi - Physiology Studies

	Summer 1998[1]											
	Beaver[2]						Mac Forest					
	98[3]		97		96		98		97		96	
Measure[4]	N[5]	S	N	S	N	S	N	S	N	S	N	S
Pseudo	1.7[a]	0.1[a]	31.7[c]	34.3[c]	56.2[d]	-	0.1[a]	0.0[a]	2.9[b]	2.7[b]	1.4[a]	0.5[a]
Erg	6.6[a]	12.5[ab]	16.5[b]	35.1[d]	23.4[c]	-	*	*	7.8[a]	15.7[b]	9.5[a]	12.7[ab]

[1]Sample date; each mean represents 2 monthly samples (June, July) from 1 branch from six trees at each site. Values of "-" indicate no foliage was available for analysis. Values of "*" indicate incomplete analysis at this time. Means with different letters are significantly different at p<0.05.

[2]Site name.

[3] Foliage age class (i.e., year of initiation).

[4]Fungal measurement; Pseudo=percent of stomata with visible *Phaeocryptopus gaeumannii* pseudothecia; Erg=ergosterol, ug / g DW needles (not species specific, represents *P. gaeumannii* and any other foliar fungi).

[5] N=north-facing slope; S=south-facing slope.

Project 4: Interaction between PG Infection and Environmental Conditions

Re: Goal 4

Methods

The goal of this project will be addressed by similar methods as outlined in project 3. However, by analyzing the diurnal gas exchange data in relation to environmental data we can determine how environmental conditions interact with SNC. At each site we are constantly monitoring light (photosynthetically active radiation), temperature, and humidity levels at half- hour intervals with Hobo dataloggers.

Results

Figures 3&4 show net assimilation rates (photosynthesis minus respiration) versus photosynthetically active radiation. Figure 3 shows that as infection increases with needle age the rate of assimilation also decreases for all light levels. Conversely, at the Mac Forest site, where PG infection is low, there is little, if any, difference

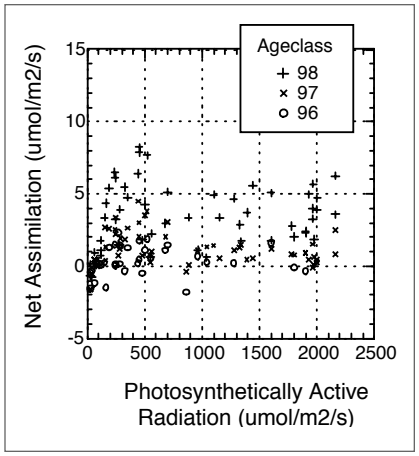

Figure 3. Net assimilation vs. photosynthetically active radiation at a heavily infected site at Beaver, OR.

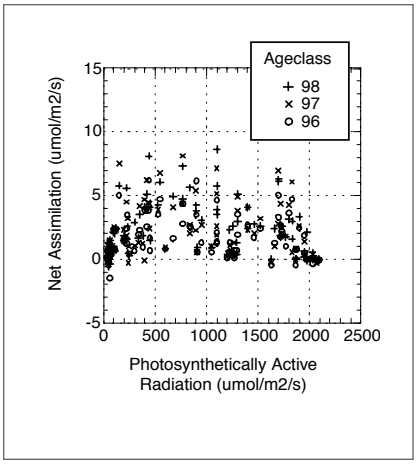

Figure 4. Net assimilation vs photosynthetically active radiation at a lightly infected site near Corvallis, OR.

between age classes (Fig. 4). By determining the light response curve, or the upper boundary of the presented scatterplots (points below the maximum are limited by other factors such as temperature and humidity) it will be possible to determine which factor(s) may be interacting with SNC to reduce foliar production. The use of such environmental response curves it will also make it possible to model the effects of SNC in any given environment. Such a tool can help forest managers determine which environments Douglas-fir should be planted in to minimize the impacts of SNC.

Project 5: Seedling Measurements of Diurnal Gas Exchange

Methods

In July 1997, 40 Douglas-fir seedlings were inoculated by spraying their branches with a suspension of ground mycelium in water. Seedlings were then left some days inside moisture chambers to protect the mycelium from drying out. 20 seedlings to be used as control were placed in chambers but no sprayed. Inoculated and non-inoculated seedlings were kept outside, and 3 seedlings per group were brought to the greenhouse for water potential and stomatal conductance measurements every month during a year. The amount of infection, needle leaf area and dry weight were determined for the needles used in stomatal conductance measurements.

Results and Further Steps

No evident differences in stomatal conductance were found between inoculated and non-inoculated seedlings. Two problems observed during the experiment might be related to this outcome. First, some non-inoculated seedlings showed infection, indicating that at least some were not disease-free before the experiment. Second, infection levels of inoculated seedlings were very low. The combination of these two factors may have been responsible for the lack of differences in stomatal conductance between groups.

Based on these results, the second year's inoculations were carried out earlier in the growing season so needles were presumably at their highest degree of susceptibility. Greenhouse-grown seedlings were used this time, to ensure the seedlings were free of *Phaeocryptopus gaeumannii.* One or two branches per seedling were covered with a plastic bag during inoculation, to keep some needles free of infection. By doing this, differences in stomatal conductance among branches of the same seedling will also be investigated.

The next period of measurements will span from October 1998 to October 1999. By the end of this period we will have not only a good understanding the physiology of infected seedlings and needles in comparison to non-infected ones, but also we will be able to learn some important aspects of fungal development after infection.

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Growth Response to Precommercial Thinning in Douglas-fir Stands with Varying Intensity of Swiss Needle Cast in the Coast range of Oregon

Status Report – Sept 1998

Alan Kanaskie, Doug Maguire, Katie Kavanagh, Mike McWilliams

Background

Many young Douglas-fir plantations in coastal Oregon exhibit extreme symptoms of Swiss needle cast disease, and these symptoms are accompanied by growth reductions. Observations suggest that thinning stands with severe Swiss needle cast may increase symptom development and exacerbate thinning shock. Other observations indicate that early thinning to maintain deep crowns may mitigate some of the growth loss attributed to Swiss needle cast. The response of stands to thinning is expected to vary according to the initial severity of Swiss needle cast at time of thinning.

Objectives

The objectives of the this study are: 1) to monitor concurrently on permanent plots the course of Swiss needle cast symptoms and the effect of the disease on the growth of individual trees; 2) to measure shifts in SNC infection severity and associated tree growth responses over time, and; 3) to measure differences in disease severity and tree growth in thinned and unthinned plots.

Methods

In April and May of 1998, twenty-three paired 0.2 acre fixed-area plots were installed in 10- to 16-year-old plantations in northwest Oregon (Figure 1). One plot in each pair was precomercially thinned to either 100 or 200 trees per acre in May, 1998. At some locations three plots were installed, with both 100 and 200 trees per acre treatments represented. All crop trees were measured for dbh, total height, height to crown base, needle retention, crown color, and crown density. Plot locations were selected across a range of disease severity classes and distributed across different topographic aspects.

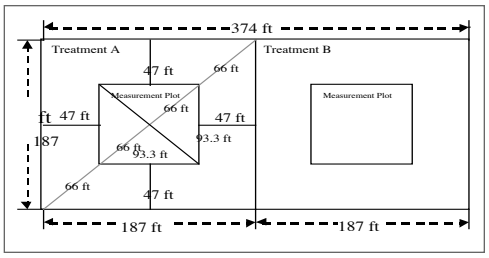

Figure 1. Example of treatment area and measurement plot for evaluating growth response to precommercial thinning in Douglas-fir stands with varying intensity of Swiss needle cast in the Coast range of Oregon.

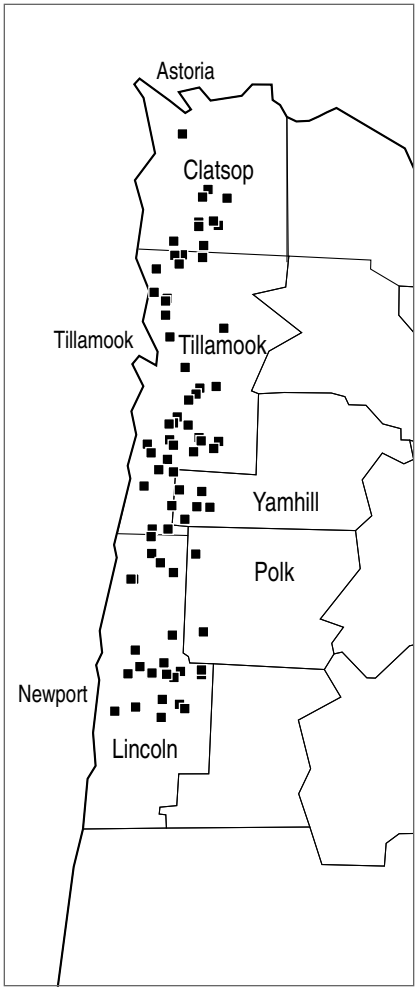

Figure 2. Location of 23 permanent plot sets to evaluate growth response to precommercial thinning in Douglas-fir stands with varying intensity of Swiss needle cast in the Coast range of Oregon.

Progress as of September 1998

The location of the twenty-three study sites are shown in Figure 2. All pre-thinning tree measurements and Swiss needle cast ratings were completed by the end of May, 1998. The data have been entered but not summarized. All plots have been thinned to specifications.

Schedule of future activities:

Activity	Year			
	1999	2000	2001	2002
Annual disease ratings	X	X	X	X
Tree measurements	X	X		

Acknowledgement

Field work was completed and coordinated by:

Steve Skinner, Matt Howard (ODF Astoria)

Steve Dutton, Jim Hines (ODF Tillamook)

Dale Anders (ODF Forest Grove)

Mike Totey, Tom O'Connor (ODF Philomath)

Rick Christian, Scott Malvitch , Bryan Capitano, Chet Smith, Michael West (ODF Salem)

Mark Gourley (Satrker Forests)

John Washburn (Simpson Timber)

Rick Allen, Ted Reiss, M. Montpas (Miller Timber Services).

The Genetics of Swiss Needle Cast Tolerance

Randy Johnson and Fatih Temel

Four series of progeny tests have evaluated the possibility of breeding for resistance/tolerance to Swiss needle cast (SNC); two series had sufficient numbers of test sites and families to allow for a reasonable analysis. The most thorough evaluation was a Northwest Tree Improvement Cooperative (NWTIC) age-11 assessment in the Nehalem area where 5 sites were evaluated for SNC/foliage traits: foliage color (1=yellow, 2=green, 3=dark green), crown density (1=sparse foliage to 6=dense foliage), and needle retention (0= <10% to 9= >90%). Age-10 data for similar traits were available from two USFS progeny test sites evaluated in the Hebo area. In both these series of trials, a subset of families was reexamined in 1998 to investigate the repeatability of the SNC/foliage scoring over time.

The SNC traits were found to be under moderate genetic control. The genetic component of individual tree variation accounted for 30% of the variation in height and DBH (i.e. heritability of 0.30), 17% of the variation in foliage color and needle retention, and 25% of the variation in crown density. The lower estimates of heritability for the foliage traits are probably a function of the difficulty in estimating the subjective foliage scores. Heights and diameters are relatively straightforward to measure; the foliage scores are all subjective and can vary much more. These data suggest that gains from breeding for SNC traits will be similar, but somewhat less, than gains possible for growth.

Resistance/tolerance to SNC involves continued growth in the presence of increased disease pressure. Past growth on the diseased sites is one indicator of future growth and the SNC/foliage traits may be other indicator traits. If an SNC trait is correlated with past growth, there would be some basis to imply that it is a possible indicator of tolerance in the future. The Nehalem data indicated that both foliage color and crown density were correlated with DBH at age-11 and height increment from age 5 to 11 (Table 1). The USFS data was more difficult to interpret because data from the two sites gave conflicting results for height growth (DBH was not assessed). The SNC traits were somewhat correlated with height growth at the moderately infected site, but there was no meaningful positive correlation at all (and sometimes negative) between SNC traits and height growth at the severely infected site. This suggests that the genetic resistance mechanisms may not function on severely impacted SNC sites. On the severely inpacted sites it is advised to switch species; don't waste time and resources trying to use genetically improved Douglas-fir.

The genetic correlations between color and crown density with height and DBH in Nehalem were much stronger than the phenotypic correlations (Table 2). This implies that some of the genes which affect growth and some which affect foliage traits are either the same or closely linked. This is a good thing for breeders since breeding for one trait will also improve the other. As an example of the possible genetic improvement, the "healthiest" ten families based on growth, foliage color, and crown density were compared to the overall population. Table 3 shows the mean values for all the families and the "healthiest" ten in the Nehalem trials. Low, but positive gains, are possible in both growth and SNC traits in this series of trials. Gains can be increased by screening larger numbers of families.

None of the Nehalem progeny test sites were experiencing severe SNC impacts at the time of assessment and, as

Table 1. Family mean correlations of SNC traits with other traits for age-11 data in Nehalem and age-10 data in Hebo.

	Nehalem			USFS-Hebo		
	Color	Crown Density	Retention	Color	Crown Density	Retention
DBH	0.35	0.39	0.07	na*	na	na
Ht	0.18	0.14	-0.01	-0.02	-0.18	0.14
Ht Inc**	0.24	0.12	0.06	-0.02	-0.21	0.15
Bud burst	-0.01	-0.04	-0.16	0.28	0.1	-0.01
Latitude	0.05	-0.04	-0.07	0.02	0.1	-0.1
Departure	0	-0.1	-0.12	-0.04	0.07	-0.02
Elevation	0.07	0.08	-0.03	-0.06	0.07	-0.02

* not available

** height increment from age-5

Table 2. Phenotypic, genetic and environmental correlations among growth and foliage traits in Nehalem.

	Individual tree correlations		Genetic correlations		Environmental correlations	
	DBH	HT inc.*	DBH	HT inc.	DBH	HT inc.
Color	0.27	0.22	0.51	0.21	0.21	0.23
Crown Density	0.32	0.1	0.48	0.16	0.26	0.08
Retention	0.08	0.11	0.13	0.11	0.07	0.11

* height increment from age 5 to 11

Table 3. Trait means for the overall population and the top 10 families chosen for health and growth in the Nehalem studies.

	DBH (mm)	Height (m)	Crown Density	Color	1st year retention	2nd year retention
Population	94.8	6.82	3.84	1.89	0.66%	0.47%
Top 10	99.1	7.06	3.96	1.94	0.69%	0.53%
% increase	3.5	4.5	3.1	2.6	3.8	12.8

a result, the family performance at each site was reasonably correlated with family performance at other sites. Correlations between the two Hebo sites was poor for SNC traits. The genetic correlations between sites within Nehalem and within Hebo were 0.92 and 0.78 for height, 0.87 and 0.31 for color, 0.85 and 0.39 for crown density, and 0.86 and 0.10 for needle retention.

The phenotypic correlations among the SNC traits over time were relatively weak; three Nehalem sites averaged 0.23 and two Hebo sites average 0.43. The age-age genetic correlations, however, calculated over all sites in a series were high. In Nehalem, the age-age genetic correlations were 0.87, 0.68, and 1.15 for color, crown density and retention, respectively. On the USFS sites the correlations were 1.37, 1.22, and 0.81. This implies that the field data we collect is relatively stable over time.

SNC impacted DBH more than height. Height of the yellow (i.e., severely infected) trees was 89% the height of the green trees, both in Nehalem and on the USFS sites; DBH of the yellow trees in Nehalem were 83% of the green trees (Table 4). Height for crown density class 2 was 86% of class 4 in Nehalem and DBH was reduced 73% in the same comparison (Table 4). These data agree with Doug Maguire's results from the growth impact study which have shown that SNC impacts diameter growth much more than height growth.

Table 4. Height (cm) and DBH (mm) means for Color and crown density.

	Nehalem		Hebo
	Ht	DBH	Ht
Crown Density			
2	653	80	437
3	728	98	493
4	760	110	525
5	769	118	522
Color			
1	679	91	453
2	761	110	509
3	-	-	518

Ongoing Research

Additional DBH assessments are scheduled for the fall of 1998 at three test locations in the Nehalem test series. This information will be used to examine how the previous growth and SNC traits affect subsequent growth. With this data we will be able to better establish the relative weights we should apply to specific traits in an selection index to choose trees and families with tolerance to SNC.

A separate research project is using 153 families from USDA Forest Service breeding programs to try to develop early screening procedures to screen for SNC tolerance. This would allow breeders to screen for SNC tolerance quickly; thus allowing for faster rates of improvement. Most families currently under evaluation in Douglas-fir breeding programs in coastal Oregon and Washington have no field data which will allow us to assess tolerance to SNC. The size (age) and/or location (SNC severity) of most progeny tests make it impossible to assess these families for SNC tolerance. Because there are no first generation data for these programs, the only way which we can quickly incorporate SNC tolerance will be to screen the second-generation families.

The seedlings for the early screening study were sown at the USFS Dorena Tree Improvement center this year. Data on germination rates and bud set have been collected. Next spring, seedlings from the 153 families will be inoculated by placing containerized seedlings in an infected forest, using a hyphae suspension spray, and placing seedlings in a fog chamber under infected foliage. Assessment of infection levels will be done in the spring/summer of 2000.

Swiss Needle Cast Infection Biology Research

Progress Report 1998

Jeffrey Stone, Loretta Winton, Bryan Capitano, Dan Manter, Pablo Rosso, Paul Reeser, Wendy Sutton, Everett Hansen

Our lab is conducting research aimed at several aspects of Swiss needle cast of Douglas-fir. This disease currently affects over 52,611 hectares of forested lands in western Oregon and has become an increasingly important concern as a threat to a major economic forest resource and ecologically dominant forest tree species in the Oregon Coast Range and western Cascades. Research is underway to examine the biological, ecological, and physiological interactions between the pathogen, *Phaeocryptopus gaeumannii*, and Douglas-fir to help understand all the contributing causes of the current disease outbreak. The causal agent is endemic in the area, and historically has been considered a pathogen of only minor importance. One of the puzzling aspects of the disease is its current severity in many coastal plantations—the present level of disease is causing unusually high levels of defoliation and growth loss. Several possible contributing factors are being examined to account for the reasons for this, and to help develop management options to minimize future disease impact.

Our research efforts are directed in six main areas:

1 Disease impacts in field sites; Determine conditions for infection. Compare epidemiology at various sites in the Tillamook area. Three plots are maintained in each of three areas around Tillamook. Each plot cluster includes a severely or moderately diseased plantation and lightly diseased and relatively healthy units. In each plantation we are monitoring weather, spore release and tree phenology, and infection period. We have examined needle retention, tree volume and growth, and wood anatomy at each of our sites. Additional inoculation experiments and field infection experiments are underway.

2 Cooperate with PNW geneticists to describe the relationship between Douglas-fir genotypes and severity of SNC. Compare SNC development in known susceptible and resistant trees. We have installed 5 of our SNC plots in progeny test plantations. Two Forest Service families are being monitored on three Forest Service sites (Cedar North, Salal Departure, and Limestone) and two State families are being monitored at Acey Creek and Coal Creek. A preliminary analysis of these families is included in this report. Work on SNC development in susceptible and resistant trees has begun with a long term outplanting study and inoculation experiments utilizing different provenances of DF.

3 Controlled field and greenhouse inoculation studies; Several studies are underway to determine the optimum conditions for infection, host tissue colonization and disease development. Studies underway include type of inoculum, source of inoculum, host phenology, host provenance, and effects of fertilization. We are developing methods that can be used for large scale, standardized tests for susceptibility and resistance in cooperation with USFS PNW geneticists.

4 Laboratory studies on pathogen physiology, infection biology and genetics; and population biology. We continue to gather, culture, and extract DNA from *Phaeocryptopus* isolates. We are continuing to maintain and process a collection of a worldwide sample of PG populations. We have initial ITS sequences. Pathogenicity tests comparing Tillamook and other isolates have been conducted and others are in progress. Single ascospore cultures have been prepared from more than 200 isolates of *Phaeocryptopus*. DNA extraction and RAPD amplification procedures have been worked out, and new, more efficient protocols are now being used.

5 Methods for detection and quantification. We have completed evaluation of detection methods based on needle clearing, epifluorescence and light microscopy of needle surfaces, and direct isolation of the fungus. We have completed development of PG-specific DNA probes for infection detection and quantification. Further observations on initial infection and needle colonization by the pathogen are reported here. We have also been working to develop more effective means for quantification of the fungus within foliage by means of ergosterol analysis. We are developing methods to use digital microscopy and image analysis. These methods are being developed to augment our

analyses and to provide research tools for cooperating researchers.

6 Landscape level analysis of disease severity and spread. Preliminary results are reported on risk analysis of Swiss needle cast in western Oregon. Further analyses with remote sensing technology are in progress.

Summary of Specific Studies in Progress

Field studies

Monitoring of tree growth, climatic factors, and disease effects in nine sites in coastal Oregon.

1. temperature, humidity and rainfall data for each site
2. tree height and caliper
3. needle retention, canopy density, transparency, discoloration rating
4. assessment of pseudothecium production on field collected branches
5. comparisons of site factors in disease severity
6. comparisons of seedling provenance/family group in disease impact

Observations on timing of field infection:

1. bagging (pathogen exclusion) experiments
2. trap tree (exposure timing) experiments

Monitoring of timing ascospore release and concentration

1. ascospore release ("spore shoot") from field collected foliage
2. weekly quantification of ascospores (spore sampling) during spring-summer

Interactions between epiphyte/endophyte fungi and PG infection

Interactions between PG and other foliage pathogens

1. Rhizoctonia needle blight

Inoculation studies

Controlled farm/greenhouse inoculations

1. Compare effects of ascospore and culture based inocula on infection/disease
2. Effects of tree phenology on infection
3. Effects of tree fertilization
4. Effects of tree source
5. Variation in virulence among PG isolates
6. Non host inoculations

Laboratory Studies

Infection Biology of PG

1. Histological studies, light and electron microsopy

Physiology of PG

1. Growth optima
2. Effects of specific growth inhibitors (e.g. melanin, chitin synthesis inhibitors)
3. Carbon and nitrogen nutrition, saprobic growth

Genetics

1. Genetic variation in geographically different isolates of PG
2. Population biology of PG
3. Phylogeny of PG

Detection/Quantification of Fungal Infection

Assessment of colonization of needles

1. pseudothecium counts
2. needle dissection and culture
3. optical and scanning electron microscopy
4. digital imaging/image analysis

Biochemical methods

1. PCR-based (nucleic acid) probe
2 Ergosterol content

Digital photography/image analysis

Landscape level analysis

1. Risk analysis
2 Remote sensing approaches

Preliminary Results

We have installed uniform weather monitoring equipment at our field sites. These include tipping bucket rain gauges, temperature and leaf wetness sensors. Weather data for the past year are being processed. The uniform equipment will allow us to make more accurate comparisons between specific conditions that vary among sites. Tipping bucket raingauges allow us to measure the number of rain episodes and their duration, in addition to cumulative precipitation. Earlier analyses showed small but consistent differences in temperature between sites, with the low elevation/higher disease sites being warmer and slightly drier much of the year (Figures 1,2,3). Rainfall is probably not limiting at any sites on the wet west side of the Coast Ranges. Spores are periodically available in the air from before budburst, and infection occurs at least through July at all plots in the Tillamook vicinity. Spores and bud burst occur earlier, spores are available longer, and infection occurs later in the season at the severely diseased Juno site than at other sites (Tables 3-5).

An earlier soil nutrient analysis and a potted seedling fertilization experiment showed no consistent effect of nutrients on pseudothecium production (Tables 6 and 7). Another fertilization experiment with potted seedlings receiving nutrient amendments is in progress at the OSU Botany and Plant Pathology farm. Some observations indicate that pseudothecium levels are consistently higher on "vigorous" trees than on adjacent chlorotic trees (Table 9). *Phaeocryptopus* may be favored on more vigorous trees, either because more nutrients are available to the internal hyphae or because more vigorous trees are able to tolerate higher infection levels. Other observations have suggested a tendency for greater numbers of pseudothecia to be produced in the upper crowns of trees. Spore germination, infection and vegetative growth of the fungus on needle surfaces is favored by a surface film of water, as supplied by dew or fog (Figures 36, 37, 38, 39, 40). This is supported by observations that there is more infection and vegetative development of the fungus (and thus lower needle retention) in the

upper crowns of trees, where dew forms and exposure to fog is greatest, than on lower branches (Table 8).

In general, there is a well supported correlation between needle retention and pseudothecium counts. On our 3 sites with more severe disease (Juno, North fork, Salal), height growth and diameter growth are reduced and the ratio of height to diameter is altered (Table 10, Figures 12, 13, 14). Average tree volume is 2.2 to 8 times greater on healthy sites than comparable diseased sites (Figure 15). The ratio of springwood to summer wood is lower on trees with poor needle retention (Figure 16).

Our studies of fungus genetics are designed to test the hypothesis that the Tillamook epidemic is the result of a unique strain or pathotype of the fungus. This work is in progress, but early results demonstrate variation in the fungus, not clearly associated with disease severity (Figures 18, 19, 20, 21). Phylogenetic analyses continue to refine our understanding of the evolution of *Phaeocryptopus gaeumannii* and its near relatives (Figure 23). Preliminary results also indicate variation in susceptibility to infection among different seed sources (Figure 17, 18) and in disease response (Figures 19, 20, 21). This also has been observed in field plots (family data are being analyzed) as well as in potted tree inoculations.

A DNA probe has been developed and is being tested for specificity to PG. The probe appears to satisfy our needs for a specific diagnostic tool to detect PG in needles and other samples that may not show other signs (pseudothecia) (Figure 24). Intensity of reaction by the probe also appears to reflect relative amounts of PG DNA present in pure preparations and tissue in preliminary experiments (Figure 25). An ergosterol based method for quantifying total fungal biomass also has been developed and can be used for estimation of infection levels (Tables 20, 21), and this measure correlates well with other measures of PG presence.

Microscopic observations of needle surfaces confirm that initial penetration of foliage occurs through the stomata (Figures 26, 27, 28). In addition to direct penetration by ascospores and internal growth of the fungus, needle colonization is also accomplished by an extensive network of vegetative hyphae that develops on needle surfaces as the season progresses and apparantly extends the infection to additional stomata (Figure 29). There are generally more surface hyphae visible on needles at sites with more disease. Analysis of biweekly trap tree exposure experiments are being analyzed. Results of some of our spore exclusion experiments indicate a biphasic infection period, with some infections occurring in the fall.

Observations of internal tissues also reveal an extensive network of fungal hyphae, but we have not seen any conclusive evidence of cell penetration. The fungus appears to absorb its nutrients form the intercellular spaces. Statistical analysis of the distribution of pseudothecia on needle surfaces shows that fruiting tends to be concentrated at needle tips.

Digital photography is a relatively new technology that has excellent applications in our project. We are using a digital microscope camera to count, measure, and record pseudothecia on needles. This provides a fast and powerful means of converting visual information to quantitative data for analysis. We are able to process more samples and extract more information from them by using this approach (Figure 41).

In related research not specifically funded by the SNCC, but supported as ancillary studies, interactions between Swiss needle cast and an apparently new foliage blight caused by *Rhizoctonia* sp. Is being investigated. A ground and aerial survey based risk analysis for Swiss needle cast is in progress, and remote sensing methods for tracking disease distribution and impact is also in progress.

Experiments in Progress

Field Plots

Nine study plots are installed in three clusters each including one plantation with moderate or severe disease, one with visible but not obviously damaging disease, and one relatively disease-free site (Tables 1, 2). Each plot has a meteorological station, with continuous recording of rainfall, temperature and leaf wetness.

Ongoing activities at each plot include:

Needles with fruiting bodies are collected at intervals and tested for spore release potential.

Needles are collected and preserved weekly for later analysis of fungus development within needle tissues. Disease level is estimated by color and retention of needles.

Height growth increment and spring wood/summer wood ratio were measured.

In four plots with visible symptoms of disease, branch tips were bagged before budburst to prevent contact with airborne spores. Bags are removed at intervals through the growing season and shoots will be evaluated later to determine periods of infection.

Potted seedling inoculum exposure experiments are underway at 3 sites.

Higher elevation plots (with less severe disease symptoms), receive more rain most months of the year than the low elevation plots (Figure 1). Through the winter months, rainfall regularly exceeded our gauges at all locations. Meteorological equipment has been upgraded at all sites

Table 1. Field plots.

	AGENCY	PREVIOUS STAND
Nehalem area		
North Fork	ODF	? alder rehab after spruce and hemlock
Coal Creek Progeny	ODF	small DF and hemlock after spruce and hemlock
Acey Creek Progeny	ODF	hemlock, spruce and DF
Tillamook area		
Juno Hill	ODF	?alder rehab after spruce and hemlock
Lower Stone Rd	ODF	DF, spruce and hemlock
Upper Stone Rd	ODF	DF
Hebo area		
Salal Departure Progeny	USFS	hemlock spruce and DF
Cedar North Progeny	USFS	DF, hemlock, and spruce
Limestone Progeny	USFS	DF

Table 2. 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 1800 ft	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 COAL CRK PROGENY	Severe	160	4.75	10	Boundary 1800FT	SW
ACEY CRK PROGENY	Moderate	220	5	10	1600FT & 1400FT	SE
SALAL PROGENY	Healthy	670	8	10	1600FT & 1400FT	E
CEDAR NORTH PROGENY	Moderate	370	4	9	1000FT	NW
LIMESTONE PROGENY	Mild	1500	7.5	9	1000FT	NW
	Healthy	890	12.25	9	1000FT	N

and made more uniform by the use of computerized temperature and wetness data loggers, and tipping bucket rain gauges. The combination temperature and wetness sensors were installed instead of relative humidity measuresments. RH is very sensitive to temperature fluctions, and very difficult to measure accurately in the biologically critical region around 100% RH (Figure 2). We are now using combination temperature-leaf wetness dataloggers at all sites to give us a more accurate and uniformly comparable measure of the critical conditions influencing spore germination and hyphal growth on needle surfaces.

Minor temperature differences between our plots are small at most measurement intervals, but remain consistent (Figure 3). Low elevation plots are 1-3 degrees warmer than the upper plots during the winter and spring, and may be a bit cooler in the summer.

Effect of Site on Disease Severity

One of our main hypotheses to explain why the disease is more severe in some areas than in others is based on geographic differences in the frequency and duration of conditions favorable for spore production and infection. These observations and experiments provide the main test of this idea.

a) Timing of spore release and variation in inoculum level among sites. Earlier attempts to use spore samplers to quantify variations in airborne spores in the field were stopped because we were unable to unambiguously differentiate the SNC pathogen from other fungi with similar spores. We are again collecting air spore samples and storing these in expectation that the DNA probe we have developed will provide a tool to differentiate and quantify PG ascospores from other fungi on the spore tapes. We have also brought needles bearing pseudothecia back to the lab at regular intervals, and induced spore discharge by suspending needles over again in petri plates (Table 3). In both years pseudothecia were mature and spores were released before bud burst at all sites. Both spore availability and bud burst occurred earlier at the low sites than at sites at higher elevation.

b) Timing of infection. Two approaches, inoculum exclusion by branch bagging and periodic inoculum exposure of potted seedlings, are being used to determine the period of infection at various test sites. These experiments were begun in 1996, and continued in 1997 and 1998.

Bagged Branches 1996. Needle infection levels were measured through the 1996 season at 6 of our test sites on bagged branches that are exposed to infection for specified periods. Twenty branches were

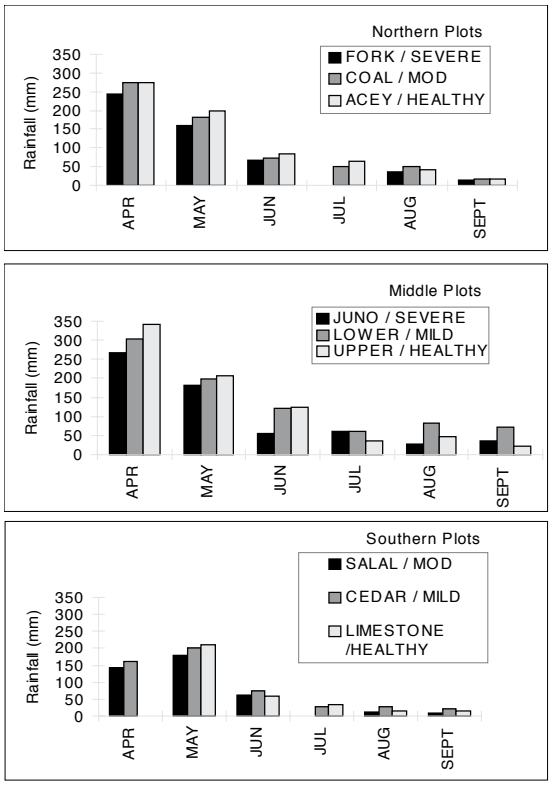

Figure 1. Rainfall at SNC plots, April - September, 1996

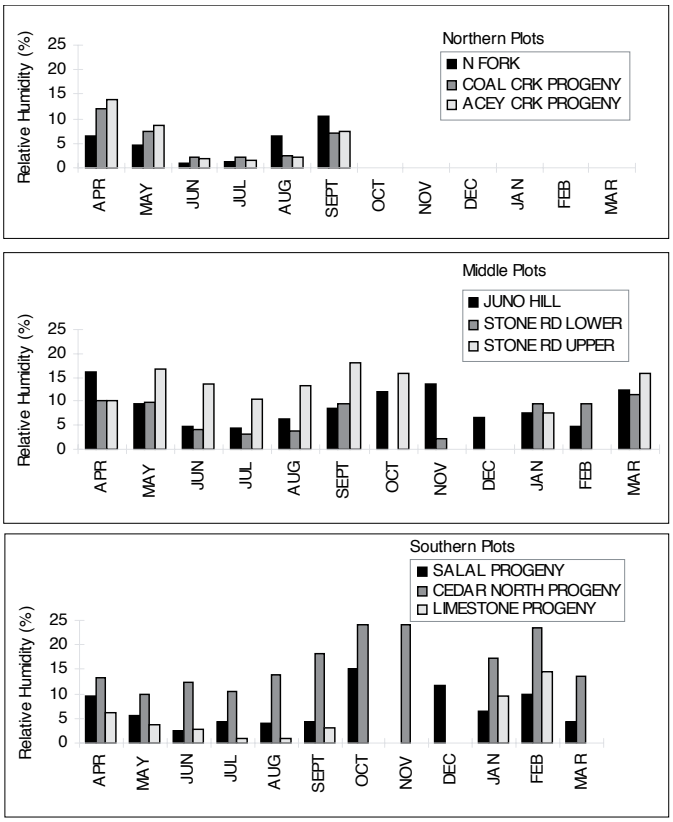

Figure 2. Relative humidity at SNC plots, April -September 1996.

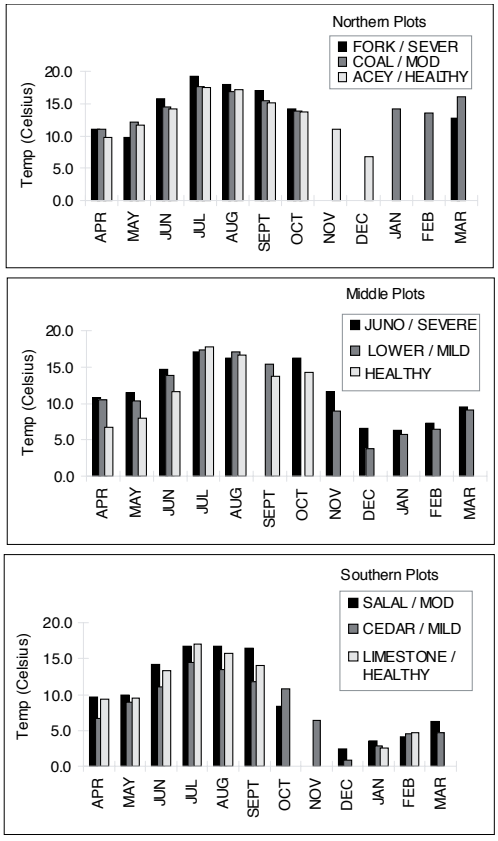

Figure 3. Temperature at the SNC plots, April 1996 - March 1997.

tagged and 18 were clipped to remove old infected needles and then bagged with pollen bags before bud burst on each of two trees at each site. Two bags are removed from each tree every two weeks, thus exposing new sets of needles to infection at intervals through the season. Pseudothecia were counted spring of 1997 on a 100 stomate sample of each of 10 needles per branch.

New infections occurred at all locations on bags first exposed after the 15th of July, and at Juno, 18 % of the needles not exposed until after the 15th of August were infected. Some infection occured at Cedar North after 1 September. Infection levels were higher at all periods at Juno than at the other sites.

Bagged Branches 1997. Branches of Douglas-fir trees were covered with translucent bags, which then were gradually removed to expose needles to varying infection periods. In 1997, two trees were selected in Salal Departure, Cedar North, North Fork and Coal Creek. In each tree 25 bags and their corresponding tags were placed on randomly chosen branches. From May to November 1997, four bags per tree were removed at different time intervals. In April 1998, all branches were removed and taken to the lab for processing. 10 needles from each branch were randomly selected and checked for presence of pseudothecia.

Figure 4 shows that the infection occurred even on branches which had their bags removed on November 21st . This means that the end of the infection period could not be detected with this experiment. The period at which most of the needles were infected, however, occurred earlier, between May and late July. These observations agree with last year's experiment.

Table 3. Tree phenology and date of pseudothecial maturity as measured by spore release from detached needles.

SITE	BUD BURST 1996 Mean	BUD BURST 1996 RANGE	END OF ELONGATION1996	FIRST SPORES 1996	FIRST SPORES 1997
JUNO HILL	28 April	17 Apr-7 May	29 June	17 April	25 March
STONE RD LOWER	12 May	1May-22May	9 July	17 April	25 May
STONE RD UPPER	25 May	7May-4June	4 Aug	7 May	25 May
NORTH FORK	7 May	24 Apr-15May	9 July	17 April	25 March
COAL CRK PROGENY	7 May	24 Apr-22May	9 July	24 April	
ACEY CRK PROGENY	19 May	7May-11June	5 July	17 April	25 May
SALAL PROGENY	7 May	1May-22May	28 July	1 May	25 March
CEDAR NORTH PROGENY	22 May	15May-29May	28 July	17 April	25 May
LIMESTONE PROGENY	22 May	7 May-29May	18 July	1 May	25 May

"High disease" plots (Salal Departure and North Fork) showed on average higher infection percentages and longer infection periods than "low disease" plots. Infection periods showed a remarkable variation from tree to tree in the same plot.

Potted Seedlings. Potted 2-year-old Douglas-fir seedlings were moved back and forth between a Corvallis site with little or no natural inoculum and the heavily infected Juno Hill site. Seedlings were potted in Juno Hill soil with or without a micronutrient fertilizer blend or in unamended soil from a healthy area inland at the summit of the Coast Range. In Corvallis, seedlings were held in the cold frames near Cordley Hall. At Juno, the seedlings were placed beneath a closed canopy of severely diseased Douglas-fir trees. We thus have a two factor test ((inoculum exposure and soil) on infection and disease development. Soils results are reported below. In the spring of 1997, 1 branch from each seedling was clipped and the pseudothecia present in a 100 stomate sample on each of 10 needles were counted. Because of the large number of zeros, results are reported as proportion of infected needles.

There was no infection on seedlings held at Corvallis throughout the year. Trees moved to Juno on May 28, about two weeks past budburst, had 71% of the needles infected, and trees that weren't moved until the 25th of June had 41% needles infected.

Exposure of groups of 20 potted seedlings for two week intervals throughout the year was initiated in 1997 and continues through 1998. Foliage from the first year's exposure are now being evaluated.

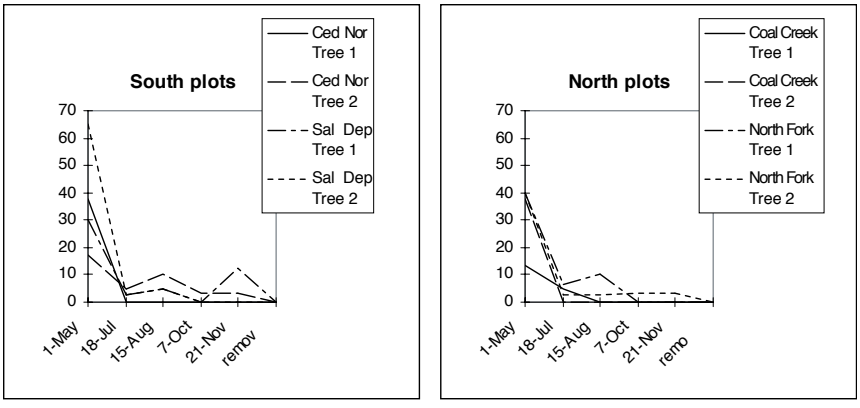

Figure 4: Percentage of needles with pseudothecia in branches that had bags removed at different times.

Effect of Soil Nutrients on Infection Levels.

We tested soil nutrition by doing a soil analysis on each of our test sites, and by fertilizing potted seedlings and exposing them to natural infection at Juno Hill. A soil sample was collected from beneath each plot tree at each of our sites, and submitted to the OSU Soils Lab for analysis. No relationship between soil nutrients (Table 6) and infection level was evident. We have initiated a new experiment with potted seedlings and fertilizer amendments at the Botany and Plant Pathology farm. These trees have been inoculated with Swiss needle cast and are being treated with a minimal fertilizer solution. Different treatments receive the minimal nutrients supplemented with different combinations and levels of nitrogen, potassium, phosphorus, calcium and magnesium. These seedlings will be evaluated for infection and disease symptom development over the next two years.

The seedlings for the exposure period experiment described above were potted in 3 different soils, including Juno soil; Juno soil plus fertilizer; and soil from a plantation located north of Sourgrass Summit at the crest of the Coast Range, that did not show symptoms of disease. The fertilizer was a micronutrient blend used in field fertilizer trials by ODF and others. There were no consistant effects of soil or fertilizer on infection level (Table 7).

TABLE 4 . Needle infection (% needles infected, or P=presence or A=absence of any infection) on branches protected from natural inoculum until different dates in 1996. (** = bags destroyed or branches dead)

SITE		DATE BAGS REMOVED								
	NO BAGS	15MAY	1JUN	15JUN	1JUL	15JUL	1AUG	15AUG	1SEP	APR'97
JUNO	82%	100%	99%	92%	93%	79%	61%	18%	**	7%
LOWER	100%	**	68%	63%	52%	22%	**	0%	**	A
SALAL	90%	100%	100%	100%	55%	22%	0%	P	**	A
CEDAR	P	**	P	P	P	P	**	P	P	A
NORTH	P	P	P	P	P	P	A	**	A	A
COAL	100%	85%	65%	100%	**	15%	20%	**	**	**

Table 5 . Infection level (% of 1996 needles infected) on potted seedlings exposed for different periods at Juno Hill or in the Cordley Hall cold frames (Corvallis).

Treatment	Exposure Period	n	Infected needles	
Plain	Corvallis throughout	36	0.0 %	
Orange	Corvallis until May 28, then Juno		31	70.6%
White	Corvallis until June 25, then Juno		33	40.6%
Green	Juno until May 28, then Corvallis		25	3.6%
Blue	Juno until June 25, then Corvallis		24	22.5%

Table 6. Soil nutrient levels at 9 sites differing in SNC severity. Sample depth is 0 to 10 inches.

		Soil Analysis					
SITE	Disease Severity	pH	P ppm	Na meq/100g	C %	N %	S %
JUNO HILL	severe						
STONE RD LOWER	mild	5	1.5	0.1	3.4	0.23	0.02
STONE RD UPPER	healthy	5.4	2.4	0.1	4.38	0.25	0.02
N FORK	severe	5.1	1.1	0.26	11.18	0.69	0.06
COAL CRK PROGENY	moderate	3.8	2.5	0.27	28.18	0.99	0.09
ACEY CRK PROGENY	healthy	4.8	2.6	0.06	6.98	0.44	0.04
SALAL PROGENY	moderate	5.1	0.8	0.06	10.18	0.57	0.07
CEDAR NORTH PROGENY	mild	5.5	4	0.18	10.48	0.57	0.05
LIMESTONE PROGENY	healthy	5.2	1.6	0.07	14.07	0.7	0.05

Within Tree and within Stand Variation in Defoliation and Pseudothecial Production

Field observations have suggested wide variation in host tree susceptibility to disease and also in the distribution of disease within the crowns of infected trees. Some observations suggest that defoliation is greater in the upper crown of many trees compared to the lower crown. We examined the top to bottom and tree-to tree variation in needle retention and pseudothecial production on current year and older needles at our field sites.

Single trees near our weather stations in 7 of our monitored plantations were cut and returned to Corvallis for examination. We measured needle retention on the ODF scale by whorl, and counted pseudothecia/ 100 stomata on a 10 needle sample at each whorl. In every tree, pseudothecial count increased and needle retention decreased with height in the tree (Table 8).

We have observed several situations where pseudothecial production is less than we expected, given the overall appearance of a stand or individual trees. We tested the relationship at the plantation at Sourgrass Summit on Highway 22, which contains trees that vary widely in appearance. Some are strikingly chlorotic, with only 1 or 2 year's needle retention, while nearby trees are dark green, and holding 2 or 3 or more years needles. On 29 May, 1997, about one week after budburst at this site, we identified 10 pairs of adjacent trees, 1 chlorotic and the other green. We noted needle retention on the 4th or 5th whorl, and collected a branch sample for pseudothecial counts (Table 9). In 9 of 10 pairs, the chlorotic tree had fewer pseudothecia than the neighboring green tree (1.8% stomata occuppied vs 4.0%). The difference was significant with a paired "t" test.

Pseudothecium production on trees in the field sites is greatest at the Juno site, the site with the greatest overall disease severity. Patterns among the sites in pseuothecium production were fairly consistent between 1996 and 1997, but with lower levels observed at all sites in 1997 compared to 1996 (Figure 5).

Table 7. Effect of soil on ratio of infected to uninfected needles, by exposure treatment (See Table 5 for treatment descriptions)

	Exposure Treatment			
Soil	Orange	White	Blue	Green
SUMMIT	2.5	0.9	0.4	0.03
JUNO	2.1	0.5	0.3	0.02
JUNO + fert	2.9	0.7	0.2	0.05

Table 8. Variation in needle retention and pseudothecial production from the 1996, 1994, and 1990 (or 1992) whorls of 1 tree from each of 7 of the OSU SNC monitoring sites.

		RETENTION (%)			PSEUDOTHECIA (%)		
SITE	Whorl	1996	1995	1994	1996	1995	1994
Upper	1996	9	-	-	2	-	-
Stone	1994	9	9	4	1	28	47
	1990	9	9	8	0	20	39
Lower	1996	9	-	-	18	-	-
Stone	1994	9	7	0	11	56	-
	1990	9	7	4	2	37	85
Juno	1996	9	-	-	56	-	-
	1994	9	0	0	50	-	-
	1990	9	0.5	0	24	88	-
Lime-	1996	8	-	-	6	-	-
stone	1994	8	5	2	5	30	67
	1992	8	5	5	0	16	6
Salal	1996	9	-	-	25	-	-
	1994	9	5	0.5	20	71	-
	1990	9	9	3	3	69	-
Acey	1996	8	-	-	0	-	-
	1994	9	8	4	1	25	45
	1990	9	9	8	0	16	13
North	1996	9	-	-	8	-	-
Fork	1994	9	0	0	6	-	-
	1990	9	4	0	2	56	-

Table 9. Pseudothecia on 1996 needles of paired yellow and green trees.

	Pseudothecia/100 stomata (1996 needles)		Needle retention (96,95,94 needles)	
Tree Pair	Yellow	Green	Yellow	Green
1	3.4	8.3	9,3,0	9,9,5
2	0.7	1.4	7,0	9,9,8
3	1.4	2.9	3,0	9,9,9
4	0.9	4.5	9,1	9,8,7
5	2.8	2.8	9,5,0	9,5,0
6	1.8	6.9	9,2,8,0	9,9,6
7	3.1	5.2	9,0,8,3	9,9,9
8	1.3	4.4	8,8,1	9,9,9
9	1.2	0.6	8,0	9,9,9,3
10	1.4	2.8	9,2,0	9,9,4
mean	1.8	4.0		

Impact of *Phaeo-cryptopus* infection on growth and needle retention of Douglas-fir.

Although the fungus is constantly associated with diseased trees, it is also present on trees with minimal symptoms of disease. It is therefore important that we make a distic-tion between the presence and the abundance of the pathogen, *Phaeocryptopus gaeumannii*, and disease severity. Put differently, presence of the pathogen is a necessary but not sufficient condition for Swiss needle cast disease. Severity of disease symptoms vary among sites and trees, and are apparently influenced by a variety of factors.

At each of our nine test sites, ten or twenty trees are marked and monitored twice each year. Growth, tree height, diameter, crown density, transparency and disease symptoms (color and needle retention) recorded. On our progeny test sites, 10 trees of each of two families are monitored.

Needle retention of one-year-old needles was similar for all sites and differences between 1998 and 1997 measurements were minor (Figure 6). Greater variation among sites is seen in the retention of two-year-old needles, with much lower values apparent at the Juno site. In general, a greater proportion of the two- year -old needles were held in 1998 than in 1997 (Figure 7). Comparisons of needle retention at all sites are compared in Figure 8. Again, needle retention at the the Juno site is notably lower than other sites; North Fork, Coal creek and Salal, all sites also have relatively poor retention of two- and three-year old foliage. Overall canopy density (Figure 9) and transparency (Figure 10) also show the most severe symptoms at the Juno site, with more minor variations among the other sites. A greater proportion of the trees at the Juno site are severely chlorotic (Figure 11).

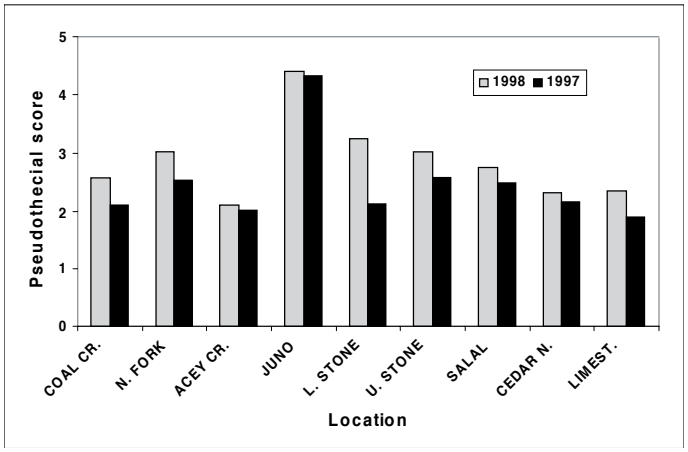

Figure 5. Comparison of pseudothecium production measured at SNC plots in 1996 and 1997.

Pseudothecium counts were also made on a sample of foliage from each annual needle complement on each tree at each site. Height increment for each of the last 5 years was measured and early wood ratio was determined for each of the monitored trees. These data are summarized in Table 10. Height and diameter growth are reduced by this disease, and the height/diameter ratio is altered. The rela-

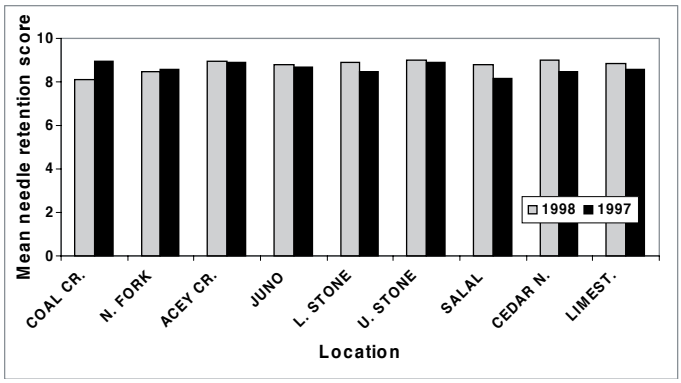

Figure 6. Comparison of one-year-old needle retention measured at SNC plots in April 1998 and April 1997.

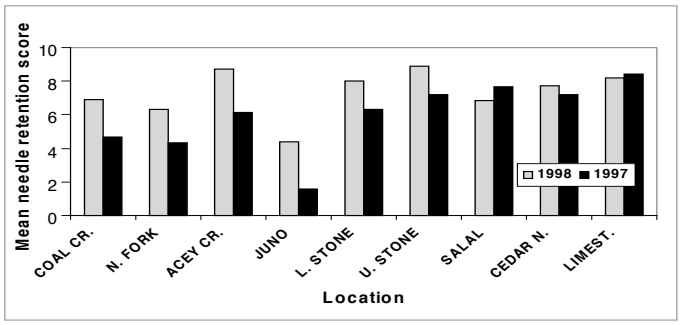

Figure 7. Comparison of two-year old needle retention measured at ANS plots in April 1998 and April 1997.

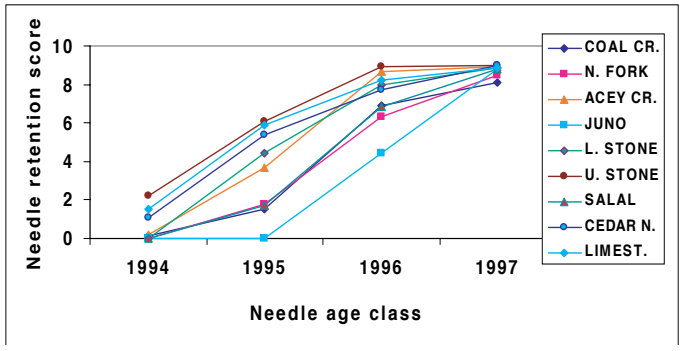

Figure 8. Comparison of needle retention over age classes measured at SNC plots in April 1998.

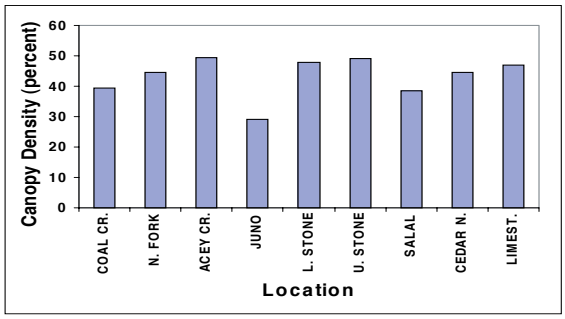

Figure 9. Canopy density measured at SNC plots, April 1998.

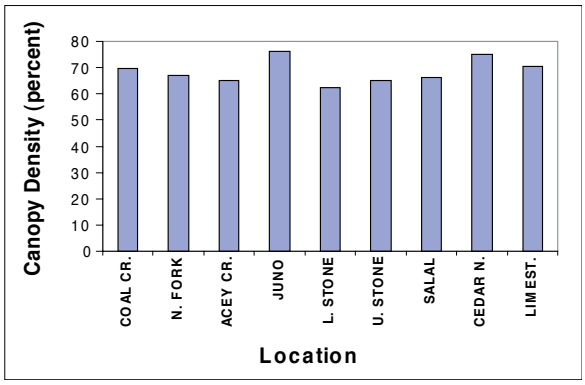

Figure 10. Foliage transparency measured at SNC plots, April 1998.

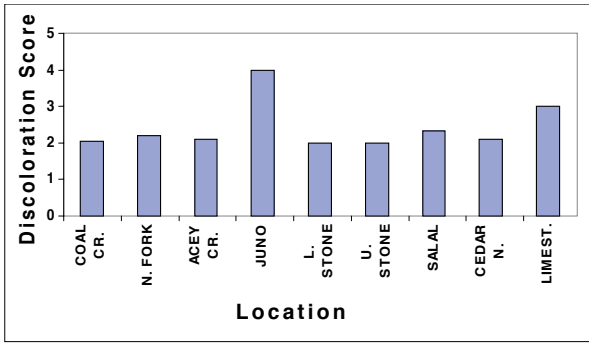

Figure 11. Foliage discoloration measured at SNC plots, April 1998. A higher discoloration score denotes greater degree of yellowing.

tionship between tree height and diameter is illustrated for 3 of our plots having symptoms rated as severe, moderate or low in Figure 12. Height to DBH is not consistent in the low to moderate disease sites, but trees at the severely diseased Juno site are consistently lower.

A comparison among sites in DBH is shown in Figure 13, and rates of growth in height increment is shown in Figure 14.

Growth rate at the Juno site is visibly affected by the disease and appears to also be reduced at the North Fork site. Tree

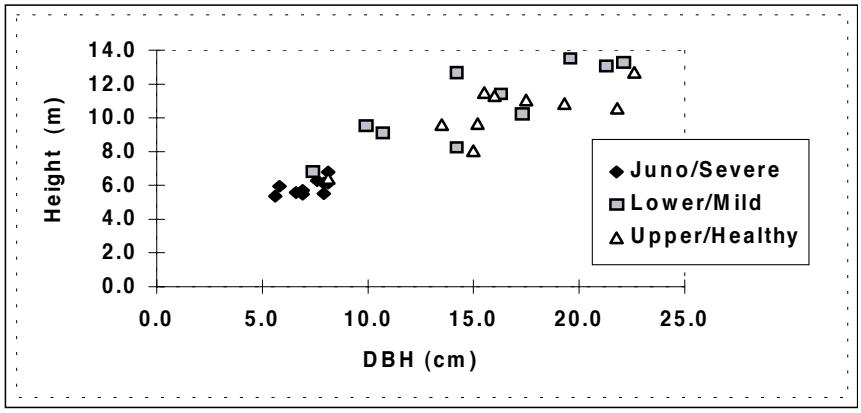

Figure 12. Relationship between tree height in 1996 and DBH for 10 trees at each of 3 sites that differ in disease severity.

Table 10. Needle retention and pseudothecial production on 1996 needles, tree height and diameter and height increment, and height to diameter ratio for plot trees at 9 sites differing in needle cast severity.

Site/Family	Family	Age	Needle Retention '96	'95	'94	% Pseudothecia	Diameter	Height Increment Height	'96	'95	'94	Hgt/Dia Ratio
N FORK												
	11	8	2.5	0	8.6	10.7	7.4	0.7	1.1	0.8	0.70	
COAL CRK PROGENY												
	3557	11	9	2.5	0	3.9	12.6	9.7	1.2	1	0.6	0.80
3513	11	9	8	3	3.0	13.9	7.5	0.8	0.9	0.5	0.50	
ACEY CRK PROGENY												
	3557	11	9	8	1.5	2.4	16.9	9.2	1.2	1.1	0.4	0.50
3513	11	9	6.5	1.5	1.7	16	10	1	0.6	0.8	0.60	
JUNO HILL		14	9	0.5	0	34.7	8	6.5	0.6	0.8	0.7	0.80
STONE RD LOWER		14	9	8.5	8	3.2	22.1	11.8	0.8	1	0.8	0.50
STONE RD UPPER		14	9	8.5	5.5	0.8	18.9	10.9	0.9	1.2	1.1	0.60
SALAL PROGENY												
	352	10	9	8.5	0.5	3.9	8.7	6.4	1	1	1.1	0.70
	426	10	7.9	8	0.3	3.1	7.3	4.7	1.2	1	0.7	0.60
CEDAR NORTH PROGENY												
	352	10	9	8.5	0	1.0	9.3	6.6	1	0.9	1	0.70
	426	10	8	9	4.5	0.8	10.7	7.1	1.2	0.8	1.1	0.70
LIMESTONE PROGENY												
	352	10	9	9	4	1.5	11.9	8.4	1.2	1.1	1.1	0.70
	426	10	8	7.5	1	0.9	9.9	7	1.1	0.9	0.8	0.70

volumes are correspondingly reduced (Figure 15). Statistical analysis of these data are in process.

Wood anatomy is also affected in diseased trees. Healthy Douglas-fir normally has much more spring wood with large, thin-walled tracheids than does summer wood, with dense, thick-walled tracheids. Trees infected with *P. gaeumannii* have this ratio reversed, there is disproportionately more summer wood than spring wood (Figure 16).

Tree Genetics

The effects of tree provenance on SNC incidence and severity, are being tested in a coordinated outplanting and inoculation experiment. Seedlings representing a range of geographic origins and genotypes planted in the northern Coast Range, and including several more distant locations, were obtained from several sources (Table 11). Seedlings were outplanted in March 1997, in a randomized design at 2 sites, one near Hebo (Evergreen) and one on Juno Hill (Beavercreek). Extra trees were planted in an adjacent plot at Evergreen (Evergreen 2) (Tables 12-14). Trees will be monitored annually for pseudothecial development. Collections from seedlings at the Beavercreek site in late September, 1998 are already showing signs of *Phaeocryptopus* pseudothecia on the current year (1998) foliage. It is very unusual to see development of pseudothecia at the end of the first growing season.

Other seedlings from the same lots were potted and inoculated in the greenhouse. Results from the first year after inoculation are shown in Figure 17. There is some small background infection apparently originating from the nurseries where the seedlings were produced. The background levels are relatively low however. Preliminary results show apparent variation among provenances with respect to infection. Several of the more resistant

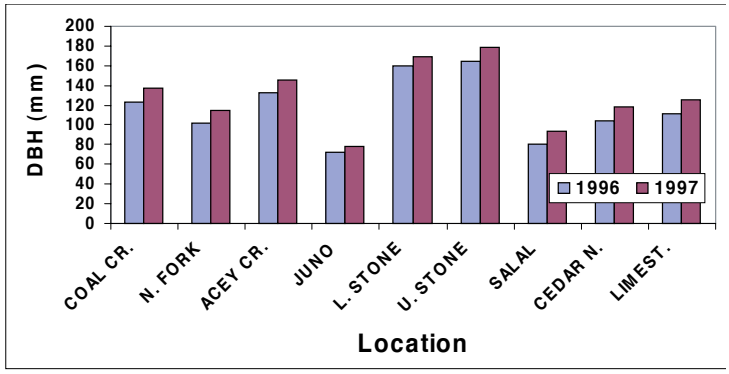

Figure 13. Comparison among sites in tree DBH.

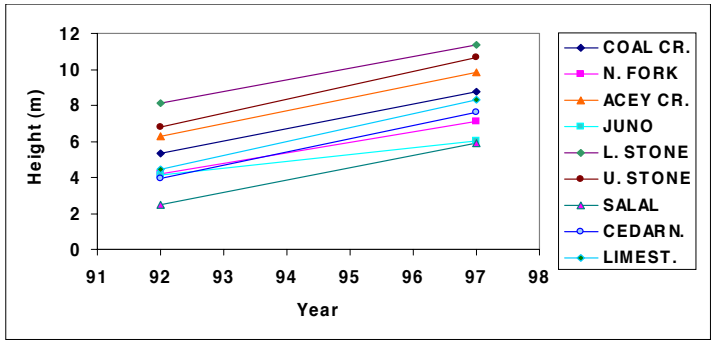

Figure 14. Comparison among sites in rate of annual height growth.

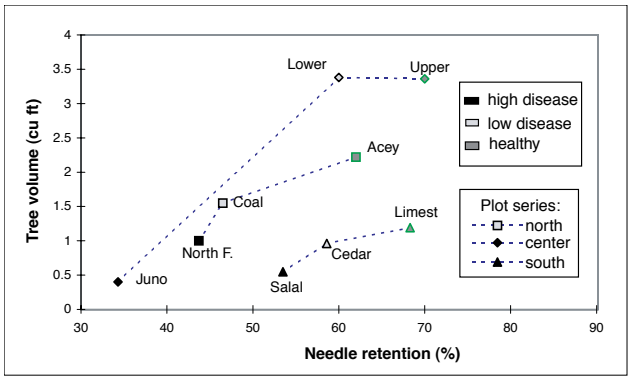

Figure 15. Tree volumes as a function of needle retention. On the x axis, three years full needle retention equals 100%, two years is 67%, and one year is 33%.

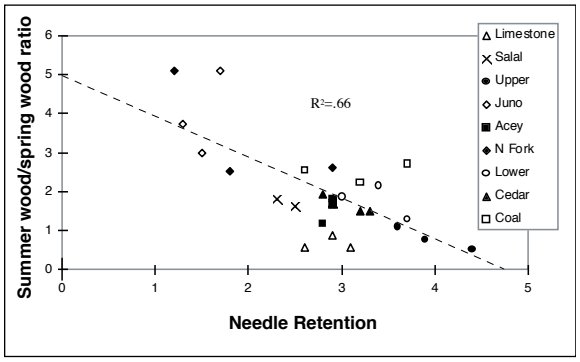

Figure 16. Summerwood to springwood ratios as a function of the number of year's needles retained by Douglas-fir trees growing at 9 sites of differing disease severity.

sources are coastal, however, no consistent patterns with respect to seedling origin are discernable.

A second series of inoculations has been initiated with a different selection of seedling proven-ances. Seedlings were inoculated at the Botany and Plant Pathology farm by applying mycelium produced in cuture and by exposure to natural ascospore release from branches collected from the field in early June and brought to Corvallis. Results will be analyzed in the next two years.

We are also monitoring the effects of disease severity, growth effects, and pseudothecium formation by tree family at field sites. Results from these measurements are being analyzed.

Genetics of *Phaeocryptopus*

The hypothesis that current disease levels may be caused by a novel, more virulent *P. gaeumannii* strain has often been postulated but has never been tested. If the Tillamook epidemic is the result of a new or unique genetic strain, then it must be either: a) a native genotype that has changed at one or a few loci, or b) an import from elsewhere. If it is an import, then we might expect significant genotypic similarities between Tillamook populations and populations in other locations experiencing severe disease such as Europe or New Zealand. On the other hand, if it is a derived native genotype, then it may be possible to find molecular markers linked to pathogenicity.

Table 11. Sources of seedlings used in outplanting and inoculation experiments.

FAMILY ID	NUMBERS	FROM	CODE	ADDITIONAL INFO	SEED SOURCE	SEED ZONE	HGT (IN)
A	11-20 to plant	GP				053-High	18.6
B	31-40 planted	GP				053-Low	18.9
C	56-70 planted	Forest Grove ODF	F.G.X-3276	H-31 1-23-97 50 PT DF)	Orchard Mix	052/1000	16.7
D	86-95 planted	Astoria ODF	B-28-70	H-31 1-23-97 50 PT DF)	Orchard Mix	051-500'	16.3
E	111-120 planted	Astoria ODF	B-27-70	H-31 1-23-97 50 PT DF)	Orchard Mix		14.4
F	136-145 planted	Philomath ODF	D-10-70		Orchard Mix	252	18.4
G	161-175 planted	FS Alsea and Waldport	65-12X-11P	202-12-12021-503-85-SB	Select Tree	061/0-1000	16.1
H	191-205 planted	FS Alsea and Waldport	64-125-09P	202-12-12021-400-0010-90SB	Orchard	061/0-1000	46.5
I	221-235 planted	FS Hebo	65-121-50T	205-12-053-01000-2.0-81S1A	Forest	053/1000	18
J	251-265 planted	FS Hebo	64-121-17P	202-12-12012-400-0515-89SB	Orchard	053/500-1500	39.6
K	281-295 planted	FS Mapleton	64-122-05P	202-12-12031-200-85-SB	Forest	062?/0-1000	43
L	311-325 planted	FS Hebo	64-121-01P	202-12-12012-400-0515-89SB	Orchard	053/500-1500	43
N	356-370 planted	Tillamook ODF	B-24	White ribbon B24	Orchard Mix	041-1000'	21.8
O	416-430 planted	Klamath ODF				701	17
P	446-455 planted	Santiam ODF				071	19
Q	471-485 planted	Coos Bay ODF				452	32.6

Table 12. Outplanting design for Beavercreek site. Letters refer to seedling lots identified in Table 11. Numbers identify location in the planting grid, and seedling number on the tag.

To plot: 0.4 mi past the Juno turnoff- take spur to right, 0.3 mi. Plot 150 ft south, past the "driftwood" pile, on the west side of the track.

81 205 H	**72** 137 F	**63** 20 A	**54** 429 O	**45** 115 E	**36** 175 G	**27** 200 H	**18** 92 D	**9** 478 Q
80 111 E	**71** 32 B	**62** 446 P	**53** 484 Q	**44**	**35** 113 E	**26** 262 J	**17** 480 Q	**8** 485 Q
79 13 A	**70** 91 D	**61** 69 C	**52** 68 C	**43** 171 G	**34** 165 G	**25** 95 D	**16** 36 B	**7** 736 F
78 366 N	**69** 294 K	**60** 370 N	**51** 483 Q	**42** 422 O	**33** 33 B	**24** 447 P	**15** 325 L	**6** 322 L
77 70 C	**68** 112 E	**59** 139 F	**50** 66 C	**41** 254 J	**032** 255 J	**023** 253 J	**14** 364 N	**5** 229 I
76 365 N	**67** 292 K	**58** 12 A	**49** 321 L	**40** 281 K	**31** 11 A	**22** 16 A	**13** 138 F	**4** 324 L
75 288 K	**66** 425 O	**57** 295 K	**48** 320 L	**39** 202 H	**30** 222 I	**21** 450 P	**12** 417 O	**3** 260 J
74 114 E	**65** 31 B	**56** 223 I	**47** 427 O	**38** 94 D	**29** 191 H	**20** 93 D	**11** 448 P	**2** 192 H
73 224 I	**64** 174 G	**55** 34 B	**46** 140 F	**37** 449 P	**28** 367 N	**19** 221 I	**10** 65 C	**1** 173 G

Table 13. Outplanting design for Evergreen 1 site. Letters refer to seedling lots identified in Table 11. Numbers identify location in the planting grid, and seedling number on the tag.

To plot: 0.8 miles north of Hebo on Hwy 101, right onto Evergreen Road. road goes through private farmyard, watch forthe electric fence at the corner of the barn. 2.1 miles to plots on right.

9 313 L	**18** 479 Q	**27** 18 A	**36** 227 I	**45** 453 P	**54** 289 K	**63** 358 N	**72** 419 O	**81** 19 A
8 319 L	**17** 323 L	**26** 116 E	**35** 454 P	**44** 117 E	**53** 317 L	**62** 172 G	**71** 258 J	**80** 455 P
7 421 O	**16** 252 J	**25** 475 Q	**34** 230 I	**43** 234 I	**52** 197 H	**61** 201 H	**70** 477 Q	**79** 357 N
6 141 F	**15** 145 F	**24** 164 G	**33** 256 J	**42** 285 K	**51** 312 L	**60** 60 C	**69** 89 D	**78** 56 C
5 39 B	**14** 58 C	**23** 119 E	**32** 120 E	**41** STUMP	**50** 204 H	**59** 428 O	**68** 452 P	77 199 H
4 143 F	**13** 423 O	**22** 362 N	**31** 363 N	**40** 90 D	**49** 142 F	**58** 369 N	**67** 87 D	**76** 59 C
3 118 E	**12** 282 K	**21** 162 G	**30** 35 B	**39** 14 A	**48** 144 F	**57** 86 D	**66** 290 K	**75** 88 D
2 15 A	**11** 163 G	**20** 482 Q	**29** 17 A	**38** 481 Q	**47** 287 K	**56** 38 B	**65** 251 J	**74** 451 P
1 235 I	**10** 203 H	**19** 40 B	**28** 57 C	**37** 161 G	**46** 418 O	**55** 257 J	**64** 232 I	**73** 37 B

To address these issues we have completed an inoculation experiment to test for differences in pathogenicity among isolates from different geographic origins, and are continuing a PCR DNA-fingerprinting technique (RAPD) to determine the possible origin of *P. gaeumannii* populations involved in the Tillamook epidemic. If significant differences in pathogenicity among strains are found, these techniques will also help to identify markers linked to pathogenicity.

Pathogenicity Tests: Experimental Design. Single-spore isolates from 4 sites (2 Tillamook severely diseased sites, 1 Tillamook mildly diseased site, and 1 mildly diseased site outside of the epidemic area) were grown in potato-dextrose broth (PDB) for 2 months. The mycelium was filtered, rinsed, weighed, and ground with a polytron homogenizer. A negative control was prepared from autoclaved mycelium. Each inoculum treatment was adjusted to .02 mg/ml dH2O, and 25 ml were applied 2 weeks after budburst in May 1997 with an airbrush paint sprayer. Treated seedlings were incubated in a mist chamber for 4 days. 400 seedlings were randomly assigned to ten treatment combinations (Table 15) including two seed sources and 5 inoculum types. In May 1998, branches were collected from each seedling and evaluated for pseudothecial development. In Sept. 1998 each seedling was measured for height, increment growth, and needle retention. Next year the seedlings will be re-evaluated.

Preliminary Results. Koch's postulates have now been completed. The inoculation method was successful and resulted in near absence of pseudothecia on the control seedlings. It is estimated that about 1% of the control needles were infected (95% confidence interval from .4% to 2%). At this point it is unknown whether this is the result of infection acquired in the nursery or control branches touching non-control branches in the mist chamber. Because we inoculated

Table 14. Outplanting design for Evergreen 2 site. Letters refer to seedling lots identified in Table 11. Numbers identify location in the planting grid, and seedling number on the tag.

EVERGREEN 2 OUTPLANTING SITE

63	**54** 265 J	**45**	**36** 359 N	**27**	**18**	**9** 196 H
62 226 I	**53** 193 H	**44**	**35**	**26** 291 K	**17** 195 H	**8** 198 H
61 473 Q	**52** 472 Q	**43** 360 N	**34** 356 N	**25**	**16** 63 C	7
60 61 C	**51** 194 H	**42** 261 J	**33** 166 G	**24** 228 I	**15** 315 L	**6** 311 L
59	**50** 476 Q	**41** 293 K	**32** 284 K	**23** 286 K	**14** 259 J	**5** 167 G
58	**49** 314 L	**40** 64 C	**31** 474 Q	**22** 368 N	**13**	**4** 318 L
57 62 C	**48** 316 L	**39**	**30** 170 G	**21** 231 I	**12** 263 J	**3** 283 K
56 169 G	**47** 264 J	**38**	**29**	**20**	**11** 233 I	**2**
55	**46**	**37** 225 I	**28** 67 C	**19** 168 G	**10** 471 Q	**1** 361 N

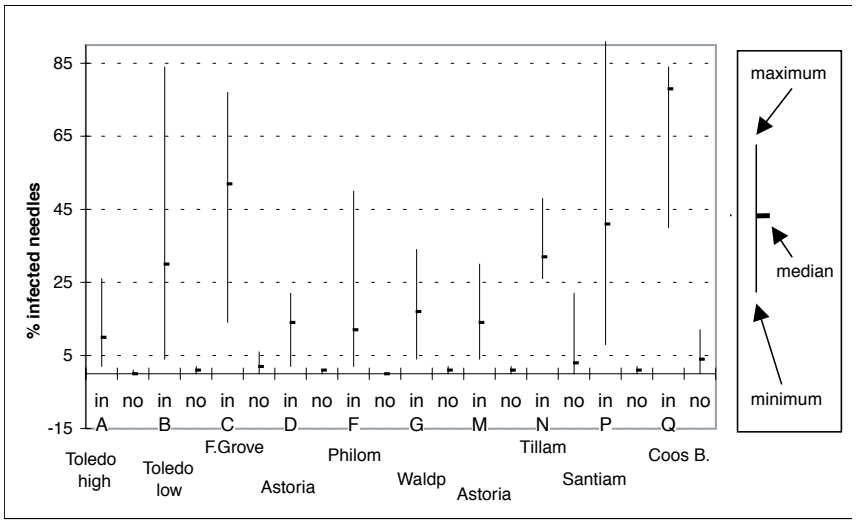

Figure 17. Median, maximum and minimum values of infection for inoculated ("in") and non-inoculated ("no") seedlings of each seed source (capital letters). Seed source locations are included under the corresponding letters.

with single-spore isolates and still obtained pseudothecia, it is probable that *P. gaeumannii* is capable of inbreeding. Because of the low amounts of infection on the control seedlings, we cannot rule out mating of inoculated strains with strains already residing in nursery infected seedlings. In a small companion study we inoculated each seedling with two different isolates. Some of these crosses resulted in higher pseudothecial formation than did the uncrossed inoculations (data not presented). This suggests that *P. gaeumannii* may also outcross. These results, although suggestive, are preliminary. The initial inoculation groups were small and the experiments need to be repeated. This work is planned to continue.

Figure 18 shows generally higher levels of infection upon the Klamath Falls seed source than the Tillamook seed source (one-sided p-value= .0001). On the Klamath seedlings, there is convincing evidence that infection level depends upon inoculum source (p-value= .0003). The Limestone and North Fork isolates produced significantly higher numbers of infected needles than did the Cascades and Juno Hill isolates. While this statement can not be made for the Tillamook seedlings, only half of them have been evaluated for infection to date. We expect to complete infected needle counts by the end of October 1998.

Keeping the present sample size disparity in mind, there is evidence of an interaction between the seed and inoculum sources (p-value=. 0011). It is interesting that, compared to the Juno Hill isolate, the North Fork isolate causes increased pseudothecial production on Klamath seedlings and reduced production on Tillamook seedlings. While this effect remains statistically questionable until all the data on Tillamook seedlings are included, there is corroborating evidence. Both of these sites are severely diseased yet pseudothecial scores (Figure 5) on field

Table 15. Treatment combinations used in pathogenicity tests. The inoculum sources were obtained from stands expressing either mild or severe SNC as judged by needle retention and color.

Seed Source	Inoculum Source	SNC Disease Level
Tillamook	Negative Control	-
	Limestone	Mild
Klamath Falls	Cascade Mountains	Mild
	North Fork	Severe
	Juno Hill	Severe

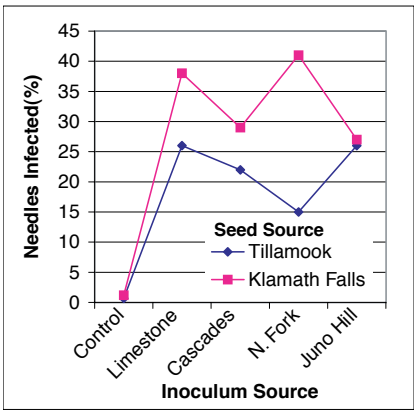

Figure 18. Mean percentage of infected needles for the 40 seedlings in each treatment combination. 100 of the 1997 needles were evaluated from each seedling.

samples from these sites reveal far fewer pseudothecia on needles from North Fork (mean pseudothecial score = 2.5) than on those from Juno Hill (mean pseudothecial score = 4.3). In addition, hyphal abundance (Table 19) on needles collected from North Fork (480 um/mm2) is much less than would be expected when compared to Juno Hill (1062 um/mm2) and the relatively healthy Acey Creek site (843 um/mm2).

Mean needle retention (Figure 19) was higher on Klamath than on Tillamook seedlings (p-value= .0001) and on both seed sources there is ample evidence that inoculated seedlings retained fewer needles than uninoculated seedlings (p-value= .0001). There is also slight evidence that needle retention depended upon inoculum source (p-value= .04) with the Juno Hill isolate causing about 10% less needle retention than isolates from the other three locations (95% confidence interval from 2% to 17%).

There is good evidence that both mean height and mean 1998 diameter increment (Figures 20 & 21) differed from the control for the Juno Hill inoculum on Klamath Falls seedlings (p-value= .0043). However we expect to see more convincing evidence when the seedlings are re-evaluated next spring.

Molecular Fungal Population Genetics: Experimental Design. So far, we have a worldwide culture collection of about 825 single-spore isolates, most of which are from the Pacific Northwest (Table 16). The samples collected from the 9 Tillamook test plots span 3 years in order to tell if fungal population structure has altered within this time as disease has increased. A preliminary study using RAPD (Random-amplified polymorphic DNA) fingerprinting as a means to estimate genetic diversity within *P. gaeumannii* has been completed. We used a small subset of our isolates for the pilot study: three populations in Washington, five in Oregon, three of which were in the Tillamook area, and two isolates from Pennsylvania.

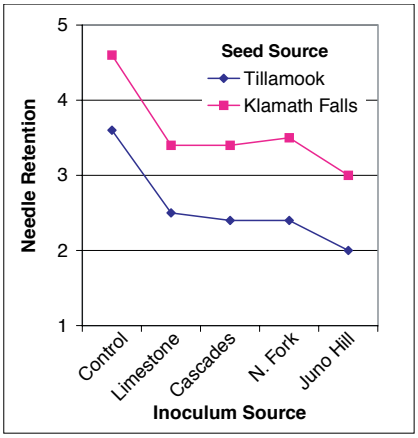

Figure 19. Mean needle retention on 1997 internodes (1=0-20%, 2=20-40%, 3=40-60%, 4=60-80%, 5=80-100%).

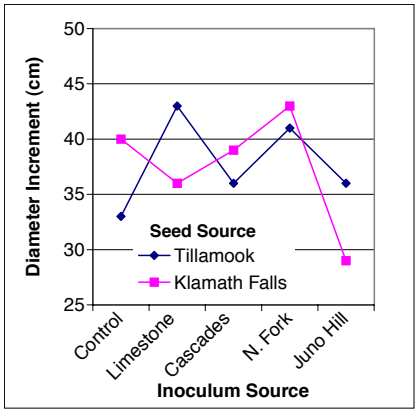

Figure 20. Mean diameter increment for the 1998 growing season.

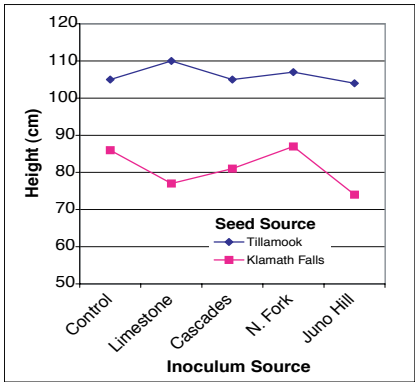

Figure 21. Mean seedling height near the end of the 1998 growing season.

Preliminary Results. In Figure 22, the length of the branches corresponds to relative similarity of the isolates fingerprints. Although the sample sizes and number of markers were too small for conclusions it is evident that there is significant genetic variability in this fungus. There are slight suggestions of

Table 16. Sample locations over three years for *P. gaeumannii* population genetic structure experiment.

1996	1997	1998
Cedar North	Cedar North	Cedar North
Salal Departure	Salal Departure	Salal Departure
Acey Creek	Acey Creek	Acey Creek
North Fork	North Fork	North Fork
Coal Creek	Coal Creek	Coal Creek
Limestone	Limestone	Limestone
Juno Hill	Juno Hill	Juno Hill
Upper Stone	Upper Stone	Upper Stone
Lower Stone	Lower Stone	Lower Stone
Sequim, WA	Mature stand near Coal Creek	Spokane WA
Forks, WA	Mature stand near Limestone	San Juan Island, WA
Pennsylvania	Mature stand near Upper Stone	
	Mature stand near Juno Hill	
	Near IFA Canby, OR	
	Near IFA Fipps, OR	
	Near IFA Toledo, WA	
	Corvallis, OR	
	Gold Beach, OR	
	Sweet Home, OR	
	Menagerie Wilderness, OR	
	Drift Creek Wilderness, OR	
	Hoquiam, WA	
	Forks, WA	
	Olympia, WA	
	Vermont	
	New York	
	New Mexico	
	England	
	Germany	
	France	
	Italy	
	Switzerland	
	New Zealand North Island	
	New Zealand South Island	

geographical clustering but no discernable correlation with pathogenicity. We are continuing this approach by increasing the sample size and number of markers. This study will be completed this winter 1999.

Anamorphic States. It has often been suggested in the literature that *Rhizosphaera*, an asexual foliar pathogen that occurs on several conifers, is the anamorph (asexual state) of *Phaeocryptopus*. This may be suggested in part because the two fungi may be found on the same needles, because they are very similar in appearance on infected needles, and because both produce fruit bodies that emerge through the stomatal pores. Despite the apparent similarities, we were skeptical of this claim because not only do the two fungi grow very differently in culture, but close relatives to *Phaeocryptopus* have hyphomycetous anamorphs which produce conidia on unprotected conidiophores. *Rhizosphaera*, however, is a coelomycete, and produces conidia within a protective structure. In addition *Rhizosphaera* occurs on several conifer genera, including pines, spruce, and true firs in addition to Douglas-fir, whereas *P. gaeumannii* occurs only on Douglas-fir. Using cladistic analysis of ribosomal DNA sequences, we have shown that *Phaeocryptopus* and *Rhizosphaera* are not closely related (Figure 23) and therefore not different states of the same organism. These results also show *Phaeocryptopus* to be a very well delimited genus, with the different isolates forming a well supported and monophyletic cluster.

Methods to Detect and Quantify the Fungus in Needles

DNA Probe. Improved methods to detect and quantify infection by *P. gaeumannii* will facilitate many studies already in progress or proposed by coop members for investigations on the effects of foliage infection and colonization by *P. gaeumannii*. It is now possible to quickly detect the presence of *P. gaeumannii* and to assess the total amount of *P. gaeumannii* DNA present within and on the surfaces of needles at any time of year regardless of the presence of pseudothecia. This should provide a very sensitive, standardized method for comparing total *P. gaeumannii* DNA within infected foliage that can be used in a number of planned or already in progress studies.

How it works. The probe is made highly specific to a short segment of *P. gaeumannii* DNA by first screening for short segments of DNA that are unique to *P. gaeumannii*. Next, a large number of copies that match exactly the sequence of bases of the *P. gaeumannii* DNA is produced. By linking these copies to a dye or radioactive label and applying it to a DNA extract from a sample, the DNA probe will adhere only to segments of DNA that have the complementary sequence and will easily wash off non

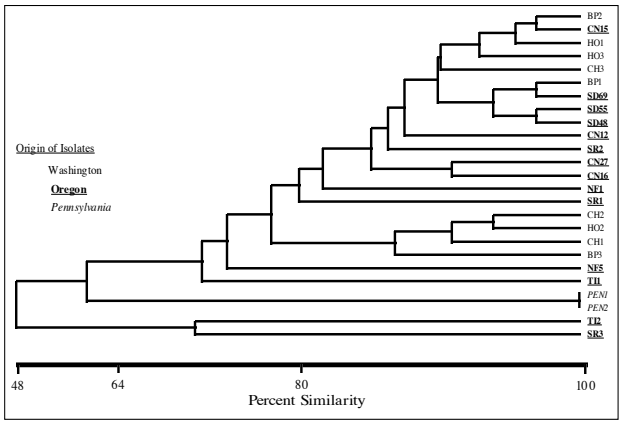

Figure 22. UPGMA phenogram for 17 markers of preliminary RAPD data. Isolates from Oregon are underlined, those from Pennsylvannia are italisized, and those from Washington are in normal font. The data were analyzed in NT-SYS using a simple-matching coefficient for the similarity matrix.

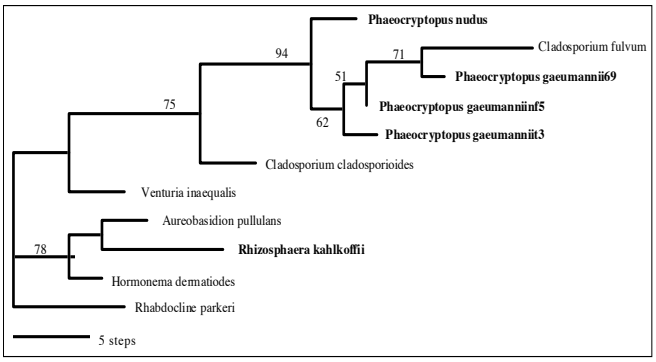

Figure 23. Single most parsimonious tree generated by branch-and-bound weighted parsimony (132 steps) of ribosomal DNA small subunit sequences (PAUP). R. parkeri *is the designated outgroup. Bootstrap values equal to or greater than 50% are displayed at the nodes. The scale bar represents the number of steps along the branches.*

complementary sequences. Selective binding should occur only in the DNA that came from the fungus *P. gaeumannii* and will be in proportion to the amount of *P. gaeumannii* present. Thus, the amount of dye visible adhering to a sample of DNA from a needle will be proportional to the amount of *P. gaeumannii* present.

Experimental Design. DNA was extracted from pure cultures of *P. gaeumannii* and several other Douglas-fir endophytes. Each sample was subjected to RAPD PCR that amplifies arbitrary sequences throughout the genome. The reaction products were examined side-by-side on a gel in order to identify a band that is specific for *P. gaeumannii*. The candidate 900 base-pair band was excised from the gel and purified from the agarose matrix. The purified PCR product was labeled non-radioactively and hybridized to a nylon membrane that had sample DNA permanently bound to it (a dot-blot). To test the specificity of the probe, a dot-blot was prepared that contained DNA extracts of each of the fungal species used to identify the candidate probe as well as total genomic DNA of uninfected, newly flushed Douglas-fir needles. To test the sensitivity of the probe, another dot-blot was prepared in which dots had increasing amounts of *P. gaeumannii* DNA as well as dots with constant amounts of the total DNA extracted from infected Douglas-fir needles.

Preliminary Results. When tested against a variety of purified DNA from fungi commonly isolated or found on Douglas-fir, the 900-base probe showed good specificity for *P. gaeumannii* (Figure 24) but also hybridized weakly to the fungus *Rasutoria pseudotsugae*, a close relaive of *P. gaeumannii*. Other near relatives of *P. gaeumannii* had essentially no reaction, and the probe did not bind to purified Douglas-fir DNA, which of course would be present in any foliage sample. The probe also generated a signal on the dot containing DNA extracted from *Allantophomopsis lycopodina*, the most genetically distant fungus included in the sample. It is unlikely that this binding was due to homology of the probe with a segment of DNA within the *A. lycopodina* genome. We think it was a false positive caused by damage to the membrane during preparation of the blot. This experiment is being repeated to confirm specificity of the probe to *P. gaeumannii.*

When hybridized to blots prepared from the total DNA extracted from samples of 10 Douglas-fir needles (Figure 25), the probe detected the presence of *P. gaeumannii* DNA within needles. The intensity of the staining also was proportional to different concentrations of purified *P.*

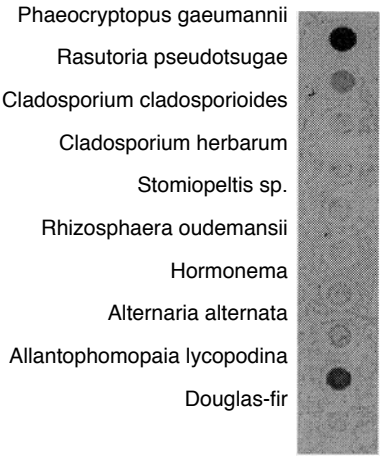

Figure 24. Dot-blot demonstrating specificity of the DNA probe. Each circle contains 1 ug of total genomic DNA. The darker the dot the more probe hybridized to the genomic DNA. However, the membrane was damaged when A. lycopodina *was applied and the signal may be an artifact.*

gaeumannii DNA and also corresponded to the level of infection indicated by the pseudothecial score. The highest levels of infection in the 1996 and 1997 Juno foliage had apparently greater amounts of DNA than were included in the test standard (purified *P. gaeumannii* DNA).

It is evident that younger needles contained less *P. gaeumannii* DNA than older needles and each of the sites differed in amount. Juno Hill, unquestionably our most severely diseased site, had very strong signals for both 1997 and 1996 needles and a detectable signal for 1998 needles, in which surface hyphae but no pseudo-

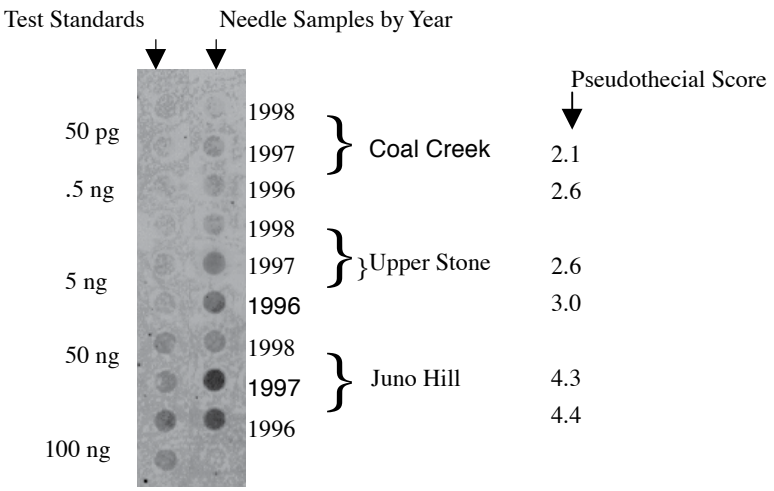

Figure 25. Dot-blot demonstrating quantitative use of the DNA probe. The first column contains increasing amounts of P. gaeumannii *DNA for use as a comparison standard. The second column contains total genomic DNA extracted from field samples of Douglas-fir needles collected from sites expressing different levels of disease (Coal Creek=moderately diseased, Upper Stone=mildly diseased, Juno Hill=severely diseased). At each site, needles were collected separately from the 1998, 1997, and 1996 internodes. Pseudothecial scores are from Figure 5 and are a visual estimate of the number of pseudothecia on needles.*

TABLE 17. Pearson Correlation Coefficients for comparisons of ergosterol content with other measures of foliage colonization by *Phaeocryptopus* and other endobiotic fungi.

	Pseudo	Erg	Phaeo	Fungi
Pseudo	1.000		-	
Erg	0.762	1.000		-
Phaeo	0.681	0.769	1.000	-
Fungi	0.766	0.818	0.849	1.000

All correlations are significant at $p<0.05$.
Data is from July 1997 samples at North Fork and Juno Hill sites.

thecia were visible. Upper Stone needles had more *P. gaeumannii* DNA for each age class than needles from Coal Creek. These data generally correspond with pseudothecial counts in foliage collected before budbreak earlier this spring (Figure 5). Future tests of the probe will use a wider range of concentrations of test standards and will refine methods of quantification of signal intensity by using densitometry and imaging software to automatically quantify sample *P. gaeumannii* DNA in needles by interpolation.

TABLE 18. Comparisons of methods for quantification of fungi from foliage samples.

	Foliage samples from July 1997[1]					
	Juno Hill			North Fork		
Measure[3]	97[4]	96	95	97	96	95
Pseudo	0.2[b]	29.9[d]	52.5[f]	0.0[a]	10.1[c]	41.7[e]
Erg	1.9[a]	16.3[c]	15.5[bc]	2.1[a]	11.1[b]	16.6[c]
Phaeo	0.7[a]	43.6[c]	44.9[c]	2.1[a]	23.4[b]	47.5[c]
Fungi	2.5[a]	71.3[c]	90.5[c]	4.0[a]	47.5[b]	83.3[c]

[1]Sample date; each mean represents 2 branches from twelve trees at each site. Means with different letters are significantly different at $p<0.05$.

[2]Site: JH=Juno Hill, low elevation; NF=North Fork, low elevation.

[3]Fungal measurement: Pseudo=percent of stomata with visible *Phaeocryptopus gaeumannii* pseudothecia; Erg=ergosterol, ug / g DW needles (not species specific, represents *P. gaeumannii* and any other foliar fungi); Phaeo=percent of foliage infected with *Phaeocryptopus gaeumannii* as determined by culture methods; Fungi=percent of foliage infected with any fungi as determined by culture methods.

[4] Foliage age class (i.e., year of initiation).

Ergosterol assay. Although not as sensitive nor as specific as the DNA probe, ergosterol can be used as a measure of total fungal biomass in foliage samples. Advantages of this method are that it is less technically difficult to use than the DNA probe, is relatively inexpensive, it is rapid and can be applied to a large number of samples. Measurement of ergosterol is less cumbersome than counting pseudothecia on individual needles, and is sensitive enough to quantify fungal biomass in needles not yet producing pseudothecia. The chief application of ergosterol analysis will be for comparative purposes. Ergosterol, a component of fungal cell membranes, can be used to assess fungal biomass in combination with pseudothecia counts or the DNA probe as a separate indication of total fungal biomass present. Measurement of ergosterol content of needles between and among trees and sites will provide an alternative quantitative measure of the levels of fungal colonization of the needles. This would be most useful in combination with pseudothecium counts, since extensive needle colonization occurs in advance of pseudothecium formation. In trees and sites with moderate to heavy colonization by *P. gaeumannii*, the relative contribution of other fungi within and on needle surfaces is probably minimal and so ergosterol can be used as an approximate measure of PG biomass.

How it works. Ergosterol is a component of fungal membranes but not plant membranes. Samples are collected and weighed and ergosterol is extracted from foliage samples by a process involving several solvents. The concentration of ergosterol is then determined by comparing the sample with a series of known concentrations of pure ergosterol run through a high performance liquid chromatography apparatus (HPLC). The HPLC separates compounds of different composition as they move through a separation column in a chemical solvent. Separated

compounds are detected according to the time it takes them to move through the column and wavelength of light they absorb. Compounds are quantified according to the amount of absorption at the peak wavelength compared to the pure standards.

Development and application of the ergosterol assay is described in detail in the report from Manter et al. Table 17 shows results from correlations of ergosterol measurements with other measures of needle colonization by *Phaeocryptopus* and other fungi. Ergosterol correlates well with *Phaeocryptopus* colonization as determined by frequency of isolation from infected needles (erg x phaeo = 0.769) and with pseudothecia counts (0.762). Correlations between ergosterol content and total fungi are somewhat higher, as expected, since ergosterol does not differentiate PG from other fungi present. Pseusothecium counts do not correlate as well with isolation frequency probably because pseudothecia do not appear on young needles infected with PG.

A comparison of measures of fungal colonization between two sites and among three foliage age classes is shown in Table 18. Ergosterol content and pseudo-thecium production on the needles are in good agreement at both sites, except that ergosterol is apparently able to detect significant needle colonization in current season foliage that has not yet begun to form pseudothecia. This is also supported by the frequency of isolation of PG in culture. As determined by ergosterol and culture methods, PG colonization reaches it maximum in 96 JH needles, but pseudothecia production is not synchronous with ergosterol content and culture frequency. Instead, maximum pseudothecium production appears delayed by one year compared with these measures of colonization.

Ergosterol content therefore seems to be a useful measure of PG infection, particularly in combination with other indicators of PG colonization. Ergosterol content correlates well with pseudothecia counts and culture method determinations of PG infection (Table 17). The contribution of other endobiotic fungi appears negligible, since in the Juno Hill samples ergosterol content stayed roughly constant between the 1996 and 1995 foliage; the frequency of non PG endobiotic fungi increased between 1996 and 1995 foliage (Table 18).

Digital microphotography/image analysis. Digital photography presents another very practical tool for estimating disease severity by quantification of pseudothecia on needles. We have been developing techniques to apply digital microphotography in combination with image analysis software for quantification of *Phaeocryptopus gaeumannii* fruit bodies on Douglas-fir needles, and for other applications in disease analysis. Counting pseudothecia on needles is a time consuming and cumbersome process. Digital microphotography/image analysis provides not only a more rapid and efficient means of quantifying numbers of pseudothecia on needles, it also enables integration of additional information, such as size distribution of pseudothecia, proportion of needle surface area covered by pseudothecia, proportion of stomata occupied, etc. Using digital imaging will allow us to collect larger samples and improve statistical reliability of our experiments and also allow a greater number of comparisons.

We can presently foresee two important applications for microphotography in the study of SNC:

1. Use light microscopy, image analysis to rapidly analyze foliage samples for the presence, distribution, and density of PG fruit bodies on Douglas-fir foliage.

2. Use light microscopy and image analysis to analyze PG ascospore concentrations from spore collector samples, possibly in combination with the DNA probe or other specific staining procedure.

We have purchased a digital microscope camera. We examined several systems that are available and found a reasonble compromise between cost and resolution. The system adapted easily to microscopes and computers currently in use in our lab. Software we are using is freely available and for now seems to serve our immediate purpose, although more elaborate image analysis programs are available at varying costs. Results so far from this configuration are very promising. An example of a digitally captured and analyzed image is shown in (Figure 26a). We are still in the process of refining the methods for using this system for routine analysis, mainly trying to improve contrast between needle background and pseudothecia so the image analysis program accurately identifies and counts pseudothecia. This system will be re ready use for analysis of a large number of specimens stored frozen.

Infection and Development of *Phaeocryptopus gaeumannii* in Douglas-fir

One explanation for the recent Swiss needle outbreak in coastal Oregon is that the coastal climate is more favorable for fungal development. Previous studies have shown that *Phaeocryptopus gaeumannii* is more abundant on trees growing in climates with wet, moderate temperatures. In few cases, however, has the fungus caused the severity of disease presently witnessed in coastal Oregon. Specifics regarding the developmental biology of *Phaeocryptopus* on Douglas-fir needles and how the environment may affect this development are poorly understood.

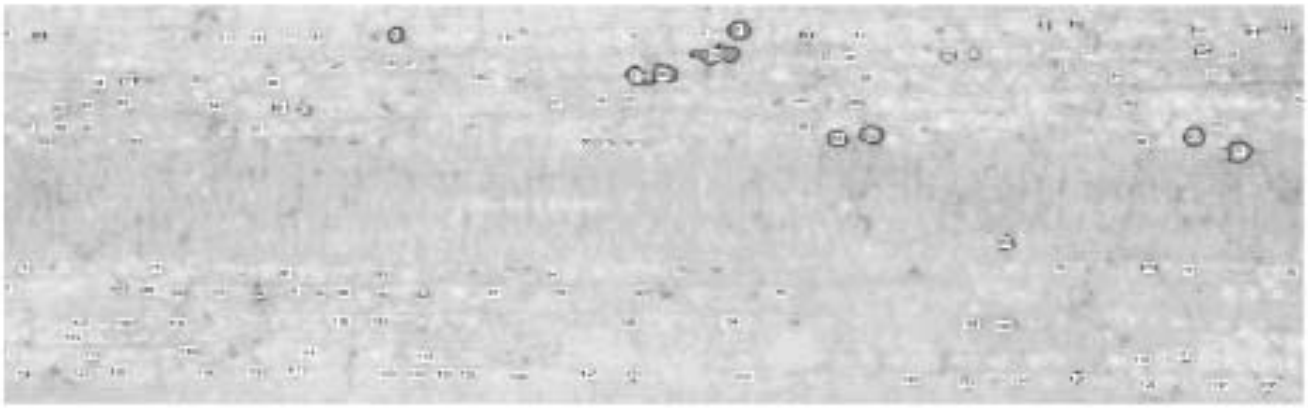

Figure 26a. Image of PG-infected needle taken with digital microscope camera and analyzed for pseudothecia. Clusters of pseudothecia are outlined, individual pseudothecia are numbered.

In particular, the mechanics of the infection process and the overall vegetative colonization ability for *P. gaeumannii* are not well worked out. Direct penetration of stomata has been suggested as the mechanism of infection but without substantial evidence. Whether needle colonization occurs at an inter- or intracellular level is still a matter of confusion, with important consequences regarding nutrition and the mode of pathogenesis for *Phaeocryptopus*. Vegetative hyphae on the surface of needles occurs frequently with this pathogen system and may exacerbate disease in some locations.

Additionally, while environmental conditions have been studied for sporulation, conditions for ascospore germination, growth, and penetration on Douglas-fir needles are still undescribed. This study aims to describe the infection and developmental biology of *P. gaeumannii* between the stages of spore germination on the needle and eventual ascocarp development. Additionally, the environmental parameters limiting fungal growth and infection of Douglas-fir needles will be addressed.

One practical aspect to better understanding the biology of *P. gaeumannii* better is that we may discover weak points in the fungus life cycle that could be targeted for disease control. For example, if vegetative hyphae on the needle surface prove an important means of colonization, then direct control of these hyphae may help check disease development. Additionally, a detailed knowledge of spore germination on needle surfaces, timing between germination and penetration, and the environmental variables controlling these events may allow more precise timing in fungicide applications where appropriate. This knowledge may also help identify particular stands or regions that favor fungal growth and are consequently at high risk for disease development.

Infection Biology, Surface and Internal Needle Colonization. Observations of the infection process and surface hyphae were made from monthly samples between summer and early winter for 1996-1997 and then in September 1997 and March 1998 for comparison and verification of the 96-97 results. First year needle samples were taken from Douglas-fir trees at three pairs of high and low disease sites in the Tillamook area: Juno Hill / Stone Upper; Salal / Limestone; North Fork / Acey Creek. Needles were collected from north facing branches of station trees at head-height and prepared with the following protocols:

Needles were painted with a thin coat of clear nail polish and allowed to dry approximately 2 hours. Dried nail polish and surface hyphae were then peeled from the needle and stained in 0.05% Trypan Blue in Lactophenol. All observations were made on first year needles from the three pairs of high vs. low disease sites mentioned. One sample is described as an observation in a field of view at 40X power under the light microscope (=0.34mm2 needle surface). For the 1996-1997 period, 50 sample observations were made for each site per month broken down by 10 observations per needle over 5 needles. Observational tallies were made of the following:

1. Length of *P. gaeumannii* hyphae per field of view at 40x.
2. Frequency of apparent penetration/ stomata in a field of view.

Similar observations were made for September 1997 and March 1998 to compare and verify results from the 96-97 season..

In addition to surface peels, needle samples were also prepared for thin section light microscopy to verify the mode of penetration into the needle. Samples were cut into 5mm segments and fixed in 3% glutaraldehyde overnight in a vacuum. Samples were then dehydrated in a graded EtOH series from 60% to 95% with 30-minute solution changes. Needle segments were then infiltrated with Historesin plastic and sectioned in 15µm cross-sections with a rotary microtome and steel knife. Sections were stained

with acid- fuschin and malachite green and mounted permanently in Permount. Sections were viewed under a compound light microscope.

Scanning Microscopy was used for observations of internal hyphae during the 96-97 as well as the 97-98 infection seasons. Needles were cut into longitudinal and cross-sections, and then fixed in 3% Glutaraldehyde in a vacuum chamber overnight. Samples were dehydrated in a graded EtOH series up to 100%, and then critical point dried, sputtercoated, and viewed with an AMRAY Scanning Electron Microscope. Observations were made on one pair of high disease/low disease sites during November 1996 — Juno Hill and Upper Stone Road. For the 97-98 season, observations were made in August 97, November 97 and then again in May 98.

Observations were made of the following:

1. The extent of internal colonization over time.
2. Whether hyphae colonize intracellular vs. intercellular regions.

Needle Penetration: Through surface peel observations, penetration of the needle appears to occur exclusively through the stoma. As spores germinate and begin growing across the needle surface (by July at most sites), hyhpae will enlarge and swell over stomata producing appressoria-like structures. Using fine focus with the microscope, a refractory point or small circle in the center of the hyphal swelling is visible in these surface peels, suggestive of an appressoria / infection hypha complex extending into the stomatal chamber. Not uncommonly, single spores will infect multiple stomata as hyphae branch after each infection and continue to grow and infect new stomata. Figure 26 is an example of multiple stomata being penetrated in successive fashion.

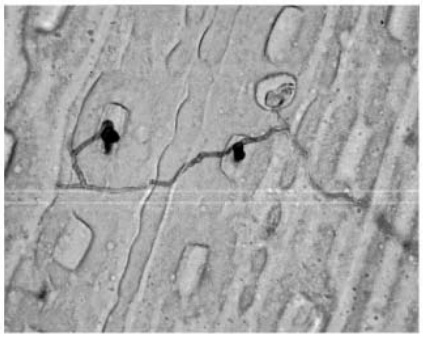

Figure 26. Successive stomatal penetration.

Thin-section light microscopy was used this last year to verify stomatal penetration. Figure 27 shows the process of appressorial formation above the stoma and a penetration hyphae inserting itself into the stomatal chamber (note the plug of hyphae in the stomatal chamber has separated somewhat from the needle surface due to sample shrinkage). Hyphae eventually grow into the needle's mesophyll interior, as depicted in Figure 28.

Penetration also occurs later in the season. By late summer / early fall, hyphae emerge from stomata and extend across the needle surface to enter adjacent stomata. Figure 29 shows an example of this late season penetration due to surface hyphae. One stomate at the bottom, right shows a condensed plug of *Phaeocryptopus* with hyphae emerging and traveling in multiple directions across the needle surface. One strand of hyphae has entered an adjacent stoma to left.

Combining early season penetration due to spores and late season penetration due to emergent hyphae, Figure 30 shows the average percent of stomata penetrated over time during the 1996-97 sampling period. Penetration begins in earnest by 28-July. Between July and November, penetration frequencies stabilize somewhat, and then increase again between November and February. Early season penetration (through July) was often associated visually with ascospores. Between November and February penetration was usually traceable to hyphae emerging from nearby stomata.

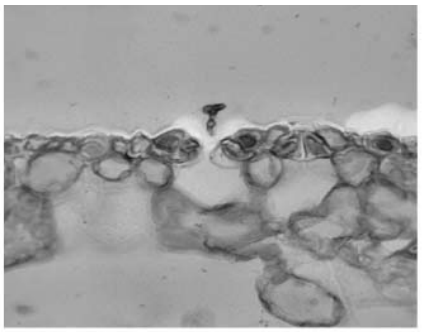

Figure 27. Appressoria and penetration hyphae entering stomatal chamber. (Juno Hill, 7/22/98)

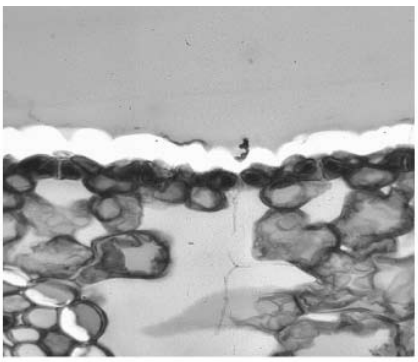

Figure 28. Germ hyphae extending from stoma into mesophyll. (Juno Hill, 9/1/98)

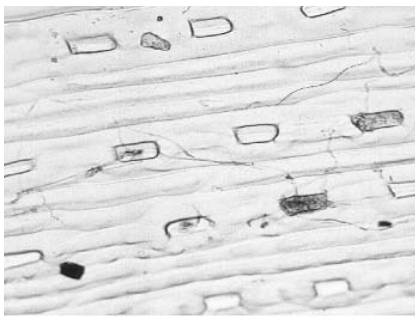

Figure 29. Late season penetration from surface hyphae.

Internal colonization: After penetration, thin-section light microscopy (TLM) and SEM reveal that *P. gaeumannii* colonizes all the major interior spaces of the needle—both the mesophyll as well as the columnar palisade region. Our original observations from Juno in November 1996 show a cobweb like mass of hyphae growing in the intercellular spaces. Observations from August 97 and November 97, so far strengthen the

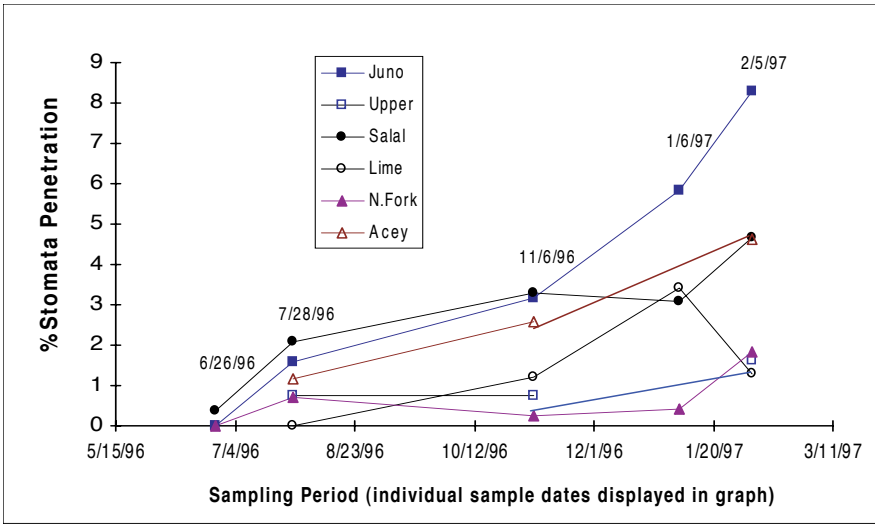

Figure 30. Percent stomata penetrated from direct microscopic observation on needle peels.

original observations that colonization is intercellular. In over 200 samples viewed with the scanning electron microscope, there were no instances of cellular penetration or intracellular growth.

Although not completely summarized yet, steady increases in hyphal abundance have been observed between the August 97 and November 97 samples. Samples from May 98 will be summarized soon.

Surface Colonization. The presence of *P. gaeumannii* hyphae on the surface of infected needles has been well established in the 1996-1997 infection season. Monthly samples from 3 pairs of high and low disease sites showed a striking trend for increase in hyphal abundance over the infection season (in terms of mean length of hyphae per surface area of needle). Over the 6-month incubation period from July to February, there was a visual increase in hyphae at all sites and a significant increase in 5 out of 6 sites (Table 19). Further, there is some suggestion that high disease sites have greater amounts of hyphae in general compared to low disease sites. For example, high disease sites Juno and Salal both had a significantly greater amount of hyphae in February than their low disease counterparts, Stone Upper and Limestone (p-values: 0.0007 and 0.0004, respectively).

Table 19 reviews the mean lengths of hyphae over the needle surface in 1996-1997. Two-sided p-values are reported from T-tests.

Samples viewed so far from the 1997-1998 period confirm the same trends. Visual increases in hyphae are apparent from all sites viewed and especially at high disease sites. Figure 31 is an example of comparisons between Juno Hill and Stone Upper for the 97-98 period. Although Stone Upper shows greater amounts of hyphae in September compared to Juno, this difference is not statistically significant (two-sided p-value= 0.48). For both Stone Upper and Juno Hill sites, there were significant increases in hyphal abundance between September 97 and March 98 (two-sided p-values: 0.043 and <0.0001, respectively). Juno showed significantly more hyphae by the end of the season than our low disease counterpart, Stone Upper (two-sided p-value=0.044), in keeping with 96-97 trends.

Table 19: (μ hyphae/mm^2 needle surface ± standard deviation; p-values

Site:	July96	Feb97	2-sample T-test
Juno Hill	94.1± 207.0	1062.7 ± 1076.2	p<0.0001
Stone Upper	61.5 ± 156.8	331.4 ± 1002.5	P=0.0657
Salal	187.2 ± 376.0	1029.5 ± 941.3	p<0.0001
Limestone	0.0 ± 0.0	402.3 ± 757.5	p=0.0005
North Fork	60.0 ± 210.0	480.8 ± 566.0	p<0.0001
Acey	128.4 ± 255.6	843.4 ± 815.0	p<0.0001

One interesting observation regarding surface hyphae is their apparent tendency for clumped distributions. General observations of surface peels revealed many samples, or areas of the needle surface, that contain no hyphae while a smaller proportion of samples contain large amounts of hyphae. The percentage of samples that had no hyphae are listed in Table 20 for the 1996-1997 season. As hyphae increase over the incubation period, however, clumping appears less distinct. This is reflected by lower percentages of samples without hyphae later in the season (by Feb 97). Similar results are listed in Table 21 for Juno and Stone Upper for the 97-98 season.

One explanation for such clumping is that one or relatively few spores are responsible for infecting a region of the needle. This small number of individuals could be responsible for the relatively defined regions of surface growth and infection observed in surface peels earlier in the season.

Temperature and Wet Period Conditions favoring Spore Germination, growth, and penetration. For temperature studies, *Phaeocryptopus* ascospores were

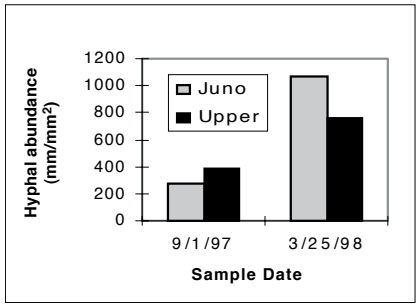

Figure 31. Comparison of surface hyphae abundance between two SNC study sites.

Table 20: Percent surface samples with no hyphal colonization, 96-97

	Juno	Upper	Salal	Lime	N.Fork	Acey
July96	72%	80%	74%	100%	90%	76%
Feb97	10%	80%	16%	62%	24%	22%

Table 21: Percent samples with no hyphae, 97-98

	Juno	Upper
Sept97	56%	78%
Mar98	8%	22%

discharged onto agar plates by pasting needles with mature pseudothecia onto petri plate lids with Vaseline above an agar surface. Petri plates were incubated in crispers with moist paper towels for one hour until sufficient numbers of ascospores were visible on the agar surface. Agar plates and ascospores were then placed on a temperature gradient plate with 4° intervals between 14°C and 34°C. Spores were observed every hour until germination began and then every 24 hours up to 72 hours. Germination percentages and total hyphal lengths were monitored over all temperature levels. A second repetition was performed with 1° interval between 18°C and 24°C.

Two experiments evaluated *P. gaeumannii* growth and infection under variable wet conditions. The first involved discharging spores in moist petri plates, as described above. Instead of casting spores onto agar, however, newly flushed and uninfected needles were placed at the bottom of the petri plates to receive spores from mature pseudothecia above. After one hour, these "newly infected" needles were placed into petri plates with either wet filter paper or dry filter paper—i.e. to simulate wet conditions vs. dry conditions. Needles under these wet vs. dry conditions were then observed every 24 hours with nail polish peels. A second repetition of this experiment introduced variable lengths of dry time by removing needles from wet plates and placing them in dry petri plates for various times—e.g. 24hr, 48hr, 72hr, and 96hr dry. These were then returned to wet plates and monitored for spore germination and resumed growth.

A second experiment evaluated the effect of wet periods on actual infection of two-year old seedlings. Seedlings were artificially inoculated with macerated mycelium. Juno isolates of *P. gaeumannii* were ground into a macerated dH2O / mycelium mixture with a homogenizer and then sprayed onto foliage with an aerosol spray gun until runoff. Seedlings were then exposed to moist conditions in a greenhouse mist chamber for specified lengths of time—0 hours (control); 1 hour; 1 day; 3 days; 5 days; 14 days. Seedlings were evaluated for amount of infection one year later with the following methods:

1. Pseudothecial counts—number of infected needles / 100
2. Culture recovery of *P. gaeumannii* from needles onto agar. Needles were surface sterilized with 95% EtOH for 1 minute, 50% Chlorox for 10 minutes, followed with 95% EtOH for another minute. Needles were then cut into 6 segments and placed onto PDMYA agar and allowed to incubate 3 weeks until *P. gaeumannii* hyphae were visible.
3. Scanning Electron Microscopy was used to visually explore the amount of internal fungi in each treatment group. Methods same as described in Methods section I. above.

Preliminary results. For the first repetition of germination and growth studies on PDA agar, spores displayed strong germination at most temperatures (Figure 32). Spores at 14°—22°C were near 100% germination throughout the 72-hour time frame while groups at 26° and 30° lagged somewhat but picked up as time elapsed. Only the 34°C group failed to germinate at any time within 72 hours. Germ hyphae displayed optimum growth at 22°C during this first experiment (Figure 33). Growth was moderate at the 18° and 26° groups. Above 26°C, growth was very poor.

A second replication of this experiment examined one-degree increments in temperature to determine more precisely which temperatures allowed optimal germination and growth (Figure 34). In contrast to the first experiment, germination was slightly more erratic at 24 hours. With the exception of some poor early success for the 24°C group, most spores germinated at 75% or better in the first day. By 48 hours and 72 hours, most temperatures allow germination to reach 100%. It is unclear why spores at 24°C showed poor germination compared to the first experiment.

As shown in Figure 35, it appears 22° and 23°C allowed the greatest overall growth of germ hyphae over 72 hours. Germ hyphae at 18°-21°C grew moderately well. The 24°C group again shows slow growth.

The effect of wet/dry period combinations on spore growth on needles

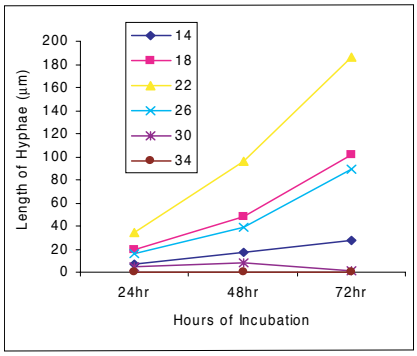

Figure 32. Germination and germ hyphal elongation at six temperatures (°C).

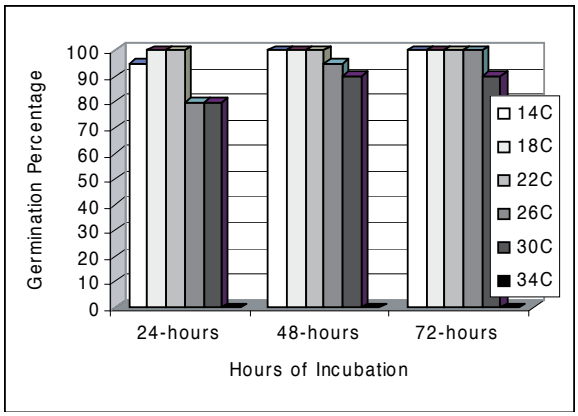

Figure 33. Percent germination between 14 – 34 C.

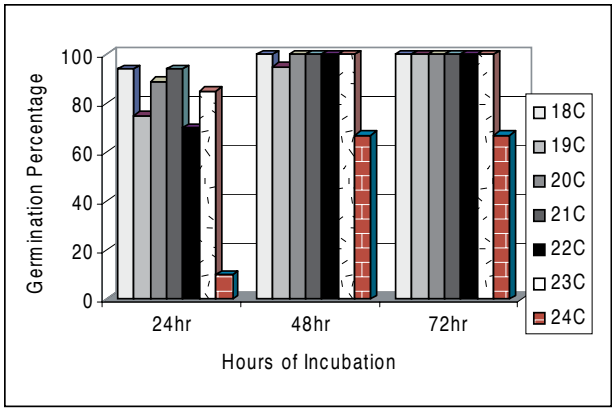

Figure 34. Spore germination at between 18 – 24 C.

showed that continuous moisture is optimal for *P. gaeumannii* growth (Figures 36 , 37). Under wet conditions, spores germinated and grew normally. Very few spores germinated under dry conditions and no measureable growth was detected.

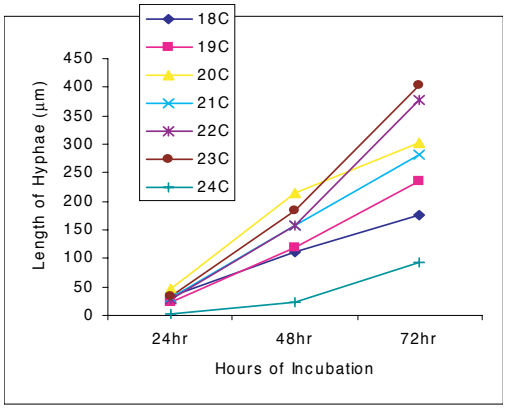

Figure 35. Elongation of germ hyphae between 18 – 24 C.

Spore germination was impaired by the introduction of a dry period following inoculation, and varied according to the length of dry period (24, 48, 72, and 96 h) (Figure 38, 39). In particular, drying for 24-48 hours resulted in drastic reductions in spore viability, both in terms of spore germination as well as growth of spores which had germinated. Spore germination after the 48-hour dry spell never climbed much beyond 55-60%. Germ hyphae growth after the 24-48 hours dry time appeared seriously compromised and did not resume the levels observed under continuously wet conditions.

The final experiment looked at the effect of varying moisture periods on the infection of artificially inoculated two-year old seedlings. After being inoculated and exposed to either 0 hours in the mist chamber (control), 1 hour, 1 day, 3 days, 5 days or 14 days, the following results were observed one year later:

A. Pseudothecia counts of 100 needles from each treatment group revealed that no pseudothecia were present. These results are surprising and not fully understood.

B. Culture recovery of *P. gaeumannii* from needles from each treatment group revealed, in general, that longer incubation periods in the mist chamber resulted in higher levels of fungal colonization (Figure 40). For the first repetition of this experiment, neither the 0-hour control or the 1-hour treatment resulted in successful colonization of the needle. However, successful infection did occur by 24-hours and each additional step in the mist chamber resulted in successively higher levels of colonization by *P. gaeumannii*. The second repetition still showed successful infection by 24-hours, however the 3 and 5 day treatments were inconsistent with repetition #1 in having lower levels of recoverable *Phaeocryptopus*. The 14-day treatment for the second repetition was consistent with the first repetition.

C. SEM observations of internal fungi from each of the treatment groups agreed with the first repetition of the culture recovery results: 0-hour and 1-hour treatments showed no internal fungi. Internal fungi were visible in the 1-day treatment in small numbers. With each increase in treatment time, SEM observations show increasing amounts of internal *Phaeocryptopus* hyphae.

Conclusions:

Needle penetration occurs exclusively through the stomata. As germ hyphae grow across the needle surface, hyphae produce appressoria above individual stoma. Thin-section light microscopy reveals that penetration hyphae are then inserted into the stomatal cavity and grow into the needle mesophyll. Multiple stomatal infections are common from a single spore. After the first infection, hyphae branch and grow in a new direction, reaching and infecting several stomata.

Penetration also occurs later in the season. By late summer / early fall, hyphae emerge from stomata, prior to pseudothecial formation, and extend across the needle surface to enter adjacent stomata.

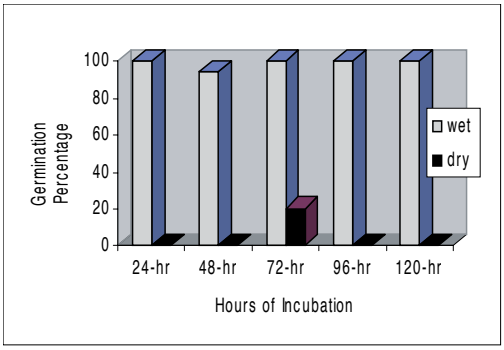

Figure 36. Germination on wet vs. dry Douglas-fir needles.

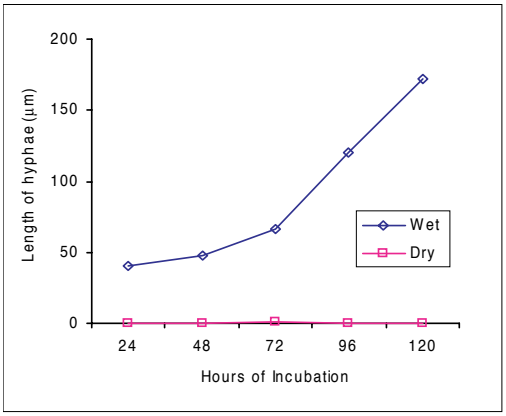

Figure 37. Elongation of germ hyphaae on wet vs. dry Douglas-fir needles.

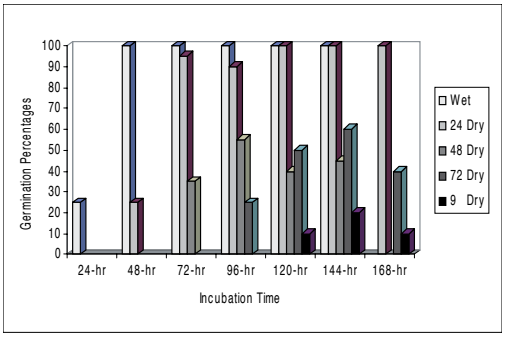

Figure 38. Effect of dry period following inoculation on spore germination.

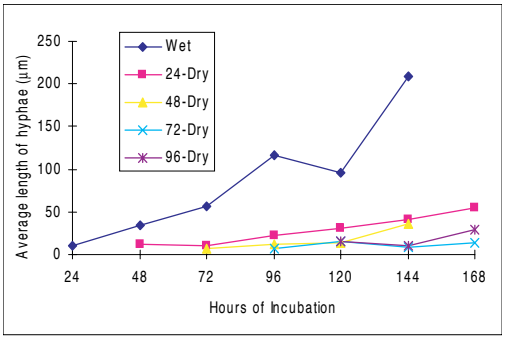

Figure 39. Effect of dry period following inoculation on elongation of germ hyphae.

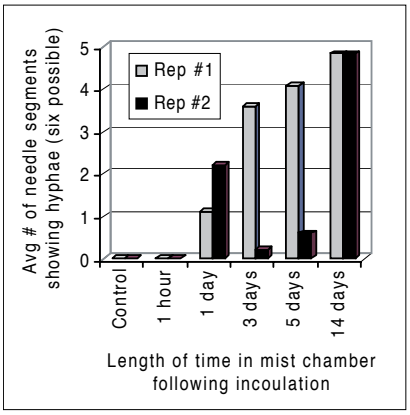

Figure 40. Recovery of P. gaeumannii *from artificially inoculated needles held in mist chamber for varying periods.*

As the infection season advances, hyphae accumulate inside the needle in both the mesophyll and columnar palisade cell region. No examples of cell penetration and intracellular growth have been observed. Growth appears strictly intercellular.

P. gaeumannii hyphae also accumulate on the needle surface. Significant increases in hyphal abundance (length / surface area of needle) were observed at most sites. High disease sites also show a trend for accumulating greater amounts of hyphae over the season than their low disease counterparts.

Optimal growth and germination temperatures for *P. gaeumannii* appear to be between 22° and 23°C. Spores grow best under continuously wet conditions while a dry spell of between 24 and 48 hours severely decreases spore viability.

According to culture recovery and SEM observations from artificially inoculated seedlings, successful infection takes place within the first 24 hours when seedlings are kept in moist conditions. Increasing times in the mist chamber resulted in higher levels of colonization.

Swiss Needle Cast Related Projects

We are also working on some Swiss needle cast related research that is not specifically funded under SNCC. The two main projects involve SNC risk analysis and remote sensing, and interactions between SNC and other foliage diseases of Douglas fir in forest and Christmas tree plantations.

A New Needle Blight on Douglas-fir

Paul Reeser and Jeff Stone

During late fall 1997 and early spring 1998 some timber growers in the Oregon Coast Range began noticing a severe needle blight in young- to middle-aged Douglas-fir timber stands. Typical symptoms appeared in current season and older growth as rapid needle death with featureless tan-brown coloration. Needles remained attached to the branchlets long after dying, which resulted in a characteristic appearance not typical of other needle diseases known in the region. Close inspection by hand lens or microscope often revealed a fine mycelial webbing over the surface of affected needles and branchlets, which apparently contributed to prolonged retention of dead needles. Buds on affected branches did not appear to be killed. Affected branches were largely defoliated by the middle of May 1998.

The needle blight on Douglas-fir appears to be a new disease, and we have found no published (or anecdotal) record of a previous occurrence. Some fairly large outbreaks of a similar disease occurred in Christmas tree plantations in Oregon during late fall 1997 and winter 1998, affecting Douglas-fir, grand fir, and noble fir. Large numbers of affected trees were rendered unmarketable due to severe blighting of large areas of peripheral foliage. The disease was first seen by the OSU Plant Clinic in Christmas tree samples received in 1996. The OSU Plant Clinic has isolated a bi-nucleate *Rhizoctonia* from diseased needles and has obtained preliminary evidence of pathogenicity on young seedling Douglas-fir. The only other report of a bi-nucleate *Rhizoctonia* causing a needle blight in conifers comes from pine nurseries in the southeast U.S. Isolates of binucleate *Rhizoctonia* have been obtained by us from blighted Douglas-fir needles collected from timber stands in Benton County in May 1998.

During the summer of 1998 we have also observed Douglas-fir trees with symptoms of the *Rhizoctonia* needle blight in timber stands in the western drainages of the Coast Range forest as far south as the Alsea River drainage in Benton County, and as far north as the Tillamook – Clatsop County line. In September we began to observe the disease invading and killing current year foliage in the lower branches.

The unique symptoms of the Douglas-fir needle blight and the absence of signs or symptoms characteristic of other known conifer needle pathogens suggest that this is a new disease of Douglas-fir. The patterns of occurrence of this needle blight observed in Oregon Coast Range Douglas-fir timber stands this year raises concern that it could have a serious impact on timber tree production if rates of spread and defoliation seen this year continue in following years.

Risk Assessment of Swiss Needle Cast of Douglas-fir in Oregon

Pablo Rosso, Jeffrey Stone and Everett Hansen and Alan Kanaskie,

Introduction and Objectives

Although the fungus that causes the disease "Swiss needle cast" of Douglas-fir has been known to occur in Coastal Oregon since 1938 (and is probably endemic), the disease has not historically been of serious concern to forestry other than occasionally on Christmas tree plantations. Recently however, a striking epidemic has developed in many Douglas-fir stands in Coastal Oregon and Washington. A method of disease survey and impact prediction is proposed here to be developed and tested in the coastal forests of Oregon.

Currently, there are few alternatives to reduce the impact of SNC; mainly, the complete replacement of affected stands by less susceptible tree species, and/or the application of fungicides. The first option is not a realistic measure in stands used for timber production because one tree species cannot easily be replaced by other species in the short run. Thus, the only options are the use of fungicides, or accepting growth loss and eventually, mortality. Although fungicides are relatively environmentally safe compared to other pesticides, their use is always undesirable because of their effects on organisms other than the pathogen.

An appropriate alternative that could increase the opportunities for manage-

ment options is to define areas of high risk of disease development. A disease risk assessment consists of determining what environmental variables are associated with the presence of the disease and then identifying the most likely areas in which disease would occur. The basis for a risk assessment is now being established using ground-based and aerial survey information.

The objective of this study is to develop a reliable, predictive disease risk assessment of Swiss needle cast in Oregon. To achieve this it is proposed to: (1) process and analyze disease distribution information, and (2) determine the environmental conditions, management practices and other factors that are associated with the occurrence of the disease in Oregon.

Methods

The risk assessment will consist of combining information about occurrence and severity of SNC with environmental and stand variables and to study the degree of association between disease and environment. It will entail two phases: a preliminary phase, based on aerial and ground survey information; and a second phase, based on satellite information, to supplement and refine the preliminary analysis. The second phase will start when the satellite-based information is available (see next section).

Geographic Information Systems (GIS) will be used to integrate, process and analyze all the information about the environment, management practices, history, etc., of the areas of interest. Application packages to be used are: Arc/Info, Arcview and GRASS. The selection of variables will be based on experience in the field, previous studies on Swiss needle cast, exploratory analyses such as the one attached, and, of course, the availability of information. Variables of interest include climate, stand management, previous vegetation composition, topography and soil. Some variables will be combined into new variables to more accurately account for differences in disease levels. For example, there is a special interest in using some type of index of fog duration, which will entail some modeling utilizing climate and topographic information.

Up to now, multivariate analysis techniques have proved to be the most effective statistical tools for the type of exploratory analysis to be initially performed in this study. These techniques permit the experimentation with several diverse variables at the same time, making the process of variable selection faster. After the most meaningful factors are selected, a model will be put together using statistical techniques such as multiple regression. Statistical packages to be used for the exploratory analyses and risk assessment model construction are PC-ORD and SAS. Model validation will be done utilizing additional information reserved for that purpose which will not be used in the model development .

Progress and Further Steps

In Appendix 1 an introductory study is presented showing the type of analysis proposed and some preliminary results. This study suggests that temperature, precipitation, altitude and distance to the coast are among the variables showing stronger association with higher levels of disease.

These variables are being reviewed and updated based on the availability of more recent disease information. Also, some progress has been made in finding a way to use fog prevalence information. As suggested by the study in Appendix 1 and by field observations, fog and low clouds may play an important role in disease development and expression.

The completion of this study will subsequently allow for the selection of low risk sites where Douglas-fir plantations could be established without the need of other intensive disease management practices. An increase in forest productivity could be obtained while maintaining a balance between local economic needs and environmental safety.

SNC and Remote Sensing

Pablo Rosso, Jeffrey Stone, Everett Hansen, Gay Bradshaw, and Maria Fiorella

Introduction and Objectives

Currently available information of the disease distribution is fragmentary at the regional scale. Ground-based disease surveying, as any intensive sampling technique, gives detailed information at the sampling unit level, but only as discrete points rather than a continuous mapping along environmental or topographic gradients. On the other hand, available information from aerial surveys is adequate to identify affected areas, but current survey data do not include Douglas-fir areas with very low disease impact. SNC research, aimed at understanding the biology and epidemiology of the disease, needs a better knowledge of, (1) extent of the landscape affected by the disease, (2) occurrence at the regional level, (3) geographic variation of disease severity, and 4) temporal variation in disease incidence and severity.

In affected stands, Swiss needle cast causes severe foliage discoloration, due mainly to needle chlorosis and defoliation. These symptoms often occur in large areas, constituting the kind of landscape features that can be readily captured by satellite imagery. Thus, satellite remote sensing appears to be a very promising technique for detecting and mapping the occurrence of Swiss needle cast at the scale of interest of this study.

The objective of this study is to develop a method of analysis of satellite imagery to map the distribution of Swiss

needle cast in Oregon. This information will then be used in the risk assessment.

Methods

Satellite images to be used are from Landsat Thematic Mapper (TM) satellite. A test area within the coastal region of Oregon will be selected using the following criteria:

(1) Presence of different levels of Swiss needle cast severity

(2) Availability of sets of clear images of at least two different years

(3) Availability of ground information for verification purposes

Different image enhancement procedures, such as contrast stretching and spectral vegetation indexes and geometric corrections, will be applied to the images as necessary and appropriate. Images will be classified using two successive approaches. First, an unsupervised classification method will be applied to identify basic land cover components. Then, different supervised procedures will be used to classify conifer stands by age.

After the areas or stands of potential Swiss needle cast impact are identified, a supervised classification based on existing ground information of the disease will be performed. A change detection method will be used as a complementary tool. This latter is based on the comparison of images from a given area at two different years. The type of cover change in the time studied will provide additional evidence for the presence of the disease. Different analyses and algorithms will be used in both types of classification procedures, based on previous, similar studies.

Additional field data will be obtained as needed, in order to calibrate the model used for classification. Groups of nearby Douglas-fir stands with contrasting spectral characteristics and classification values will be surveyed in situ to determine the correspondence between image values and the levels of defoliation and chlorosis. Test stands will be first located on aerial photographs, and depending on their accessibility, chosen for ground survey.

To evaluate the accuracy of the disease mapping, a second set of field data will be produced. Aerial photos will be used to aid in the process of classification, and to design the ground survey scheme. They will also be used as an alternative source of information to help in the classification accuracy testing.

Once the technique for disease mapping is developed, it will be applied on at least two or three more areas.

Progress and Further Steps

A satellite image has been purchased and is being analyzed. A complete and updated digital vegetation map of the Oregon coast is being acquired to help in the process of defining vegetation cover types and forest stand composition in the area of the image.

Disease distribution information derived from satellite image analysis will be used to supplement and refine the preliminary risk assessment analysis.

SNC and Seedling Provenance

Pablo Rosso and Everett Hansen

Introduction and Objectives

In determining what are the main factors that need to be considered to explain and predict the presence of disease in the field, it is necessary to understand the effect of each variable separately. The objective of this study was to preliminary assess the importance of seed source in disease expression on artificially inoculated seedlings.

Methods

A total of 140 seedlings from 10 different seed sources were used in this experiment. In July 1997, about two thirds of the seedlings were inoculated using the method presented in the previous section, and one third was left uninfected to be used as control. Seedling initial height and caliper, and previous year's apical growth was measured. Seedlings were kept outside. 1998 bud burst was recorded for each seedling. A first infection level measurement was done by the end of the summer 1998 (September 15th). A second and definitive set of measurements, including infection levels and seedling growth, will be done in summer 1999.

Preliminary Results

About one third or half of the inoculated trees of each seed source had very low values of infection or no infection at all. This can be attributable to the fact that inoculation was carried out late in the season when needles are less susceptible. Almost all provenance groups had infected non-inoculated (control) seedlings. Observations made during this experiment indicate that many seedlings were infected with *P. gaeumanii* when they were brought from the nursery. The infection recorded in control seedlings was used to estimate a "background" level of infection in all trees. Inoculated trees that had less infection than the control trees were then not considered for the computation of the statistics.

Figure 17 (page 34) shows medians, minimum and maximum values of seedling infection per each seed-source (capital letters). Besides the infection levels of artificially inoculated seedling, infection levels of control seedlings are shown. Infection was computed as percentage of needles with pseudothecia over a total of 100 needles per seedling.

Although the relatively low sample size of this study and the wide range of infection levels observed prevent this study from providing any strict evidence of differences among groups, results suggest

that seed source has an effect on infection levels. Moreover, it can be noted in Figure 17 that all coastal seed sources, Toledo high and low altitude (letters A and B), Astoria (A and B), Waldport (G) and to a lesser extent, Tillamook (N) had lower medians. Whereas interior sources, such as Forest Grove (C) and Santiam Pass (P) had high medians. This leads to the hypothesis that seed sources that had more exposure to the fungus are less prone to manifest higher infection levels. Higher values of Coos Bay origins (Q) may not be an exception to this principle given its far south location, away from the sites with higher disease incidence in the region.

Further Steps

By the end of this two-year experiment it will be determined whether or not disease responses are different among seedlings of diverse seed sources. The growth of inoculated and non-inoculated seedlings of different provenances will also be compared.

APPENDIX

Background Analysis for the Risk Assessment Project

Environmental factors associated with Swiss needle cast of Douglas-fir: A multivariate approach

I. Introduction

Swiss needle cast of Douglas-fir is a foliar disease that affects forest plantations in the coastal areas of the Pacific Northwest. The causal agent is the ascomycetous fungus *Phaeocryptopus gaeumannii*, an endemic parasitic fungus known to occur only in Douglas-fir. However, its presence and abundance seem to have increased in some areas where disease symptoms have been consistently worsening. This led to researchers to propose that under some circumstances, an excessive development of the fungus disrupts the normal functioning of the needles, causing foliage chlorosis and further defoliation.

This disease constitutes an atypical situation in which the presence of the fungus by itself does not determine the severity of disease. Disease severity is the result of a complex interaction between the development of the fungus inside and outside the tree needle, the genetic and physiological characteristics and status of the tree, and the environment.

Finding the environmental factors that are geographically associated with the presence of diseased tree stands may help to understand the most important factors in influencing disease severity. The final product of such a study, a disease risk assessment, will help in planning for future management of the problem.

The aim of this project was to apply basic multivariate statistical tools to select a set of environmental variables that could be associated with the presence of disease in the central and north coast of Oregon.

II. The dataset

A ground disease survey carried out by Oregon Department of Forestry in 1996 and 1997 provided a list of 112 stands. Each stand had disease level assigned to it, a rating system that ranges from 1 (low disease) to 6 (high), based on the appearance of the stand, and the average degree of crown defoliation and stem growth reduction. Since the pathogen is a ubiquitous parasite of Douglas-fir, virtually present in all natural populations, no strict "no disease" category exists.

A digital map of stands was overlaid on predicted precipitation and temperature grids, digital maps of past vegetation types, and digital elevation model grids (DEM's). The information on these maps and grids was manipulated, rescaled and averaged, so that each stand could be assigned a value of:

a) average annual and June daily precipitation in 1993, b) average annual and June daily maximum temperature in 1993, c) average annual and June daily minimum temperature in 1993, d) altitude and e) distance to the ocean coast.

And the following categories:

One of five possible aspects, North, East, South, West or No Aspect, and b) one of three original stand compositions, Douglas-fir, Non-Douglas-fir, or Unknown.

All geographic data management was done using Arc/Info. No specific single criterion was used to select the variables. They were chosen based on general common sense about tree pathology, previous knowledge on Swiss needle cast, and availability of information. 1993 was used as a source of climate variables because it was the most recent available year. As a final product, a single matrix was generated for the analyses below, in which environmental variables were placed in columns and sampling units (stands) in rows.

III. Questions and approaches

The main question of this work was whether or not stands with similar disease rating share similar values or categories of environmental factors and/or stand history. Methodologically, the question was approached in two different ways. First, the disease categories of the stands were assumed to constitute a natural, a priori grouping, so the existence of these groups was tested using a multi-response permutation procedure test (MRPP). In the second approach, instead of assuming the presence of disease groups, a separation of stands by groups was attempted through cluster analyses, using only the environmental variables. The resulting groups were consequently contrasted with the disease categories and the degree of matching between them was assessed. Finally, an ordination technique, a non-metric multidimensional scaling test (NMS) was used to study the distribution of stands (and their corresponding disease levels) in the environmental space.

The secondary question of this work had more to do with the way in these

and future similar analyses should be done. The two categorical variables used in this study are examples of many other factors that may have a crucial role in disease expression and that must have a qualitative structure. Analytically, these types of non scalar variables are typically treated as indicator (or "dummy") variables (Tabachnick and Fidell 1989). By doing this, however, for every single original variable (such as vegetation type) a number of new variables that equal the number of categories of the original variable minus one, are created. Since discussion of the effects of this type of data transformation could not be found in the literature, some experimentation in this direction was proposed in the present project. Specifically, the multivariate tests mentioned above were applied before and after the indicator variables were removed from the dataset and/or changed to continuous variables.

IV. Preliminary analyses: data structure

The appropriateness of the data structure was evaluated before starting the multivariate analyses. A function called "Row and column summary" in PC-ORD multivariate statistic package was used to obtain descriptive information of each environmental variable. Frequency distribution histograms of each variable were also constructed to assess the general structure of the data. Although there was no need for testing multivariate normality since only non-parametric tests were used, the univariate analysis is always useful, so unexpected values and outliers can be easily detected.

Because of obvious differences in absolute values and ranges among variables (reflected in the high Coefficient of Variation of the columns, CV = 256.7%), a general relativization by columns was done, to allow comparisons among variables on a uniform basis. The general relativization procedure with the corresponding parameter equal to 2 consists of taking each value and dividing it by the square root of the sum of squared individual values of the column. The relativization procedure reduced column CV to 38.2%. In contrast, initial row totals were reasonably homogeneous (CV=23.9%).

To check for the presence of multivariate outliers, an ad hoc function in PC-ORD was used. In this procedure, a method of calculating distances among sample units is chosen. In this study, Euclidean distance was used. This procedure identifies stands that have a value of distance in units of standard deviations (in this case, 3) greater than the average distance. No stand in this study was selected, indicating the absence of evident outliers.

V. Are disease categories "true" groups?

Multi-response Permutation Procedure test (MRPP). This procedure provides a test of hypothesis of differences among pre-determined groups. In this case, the variability of stand environmental values within and between disease groups was assessed to test for the existence of "true" groups. First, 5 disease groups of stands were used. The only stand with a disease rating of 6 was included into the 5th group, because the method does not accept groups with only one member. The 6-category disease rating system is somewhat arbitrary and probably too precise to be useful in finding general associations between environment and disease levels. For that reason, a broader disease categorization was constructed to run the MRPP analysis a second time. Disease ratings 1 and 2 were merged into one group and 3, 4 and 5, into another. This secondary grouping was based on field experience according to what an experienced observer would consider a stand "reasonably healthy" or not. For the MRPP analyses PC-ORD was used, with Euclidean distances and n/sum(n) as the weighting option.

Characteristics and results of the two tests are summarized in Table 22. The p-values of the two tests do not support rejection of the null hypothesis of no differences between groups, if a strict threshold of $p < 0.05$ is used, but are low enough to suggest a difference between groups in the second test. In addition, R-values are low compared to usual ecological studies, indicating low homogeneity within groups.

It is concluded that stands grouped by disease ratings do not constitute well delimited groups representing different values of environmental factors. This means that the set of variables chosen cannot be used to classify stands according to their disease levels.

VI. Seeking groups of stands that share environmental characteristics

Cluster analysis. To see if stands with similar disease values constitute groups with similar environmental characteristics, stands were grouped according to the latter and then disease rating was overlaid on the groups. Hierarchical clustering is a method that determines similarities among entities and aggregates these entities accordingly, establishing which one should be closer than other. In a statistical package like PC-ORD, a

Table 22. MRPP results

	Group numbers and sizes	T (test)	p (prob.)	R
1st test (5 groups)	1=4, 2=29, 3=44, 4=28, 5=07	-0.93	0.17	0.006
2nd test (2 groups)	1=33, 2=79	-1.51	0.08	0.005

distance measure method between objects and a grouping strategy is chosen. In this study Euclidean distances and Ward's method were used.

As stated in Section III, it was suspected that variables such as slope aspect, made into four indicator variables for the calculation purposes, would highly influence the grouping results, especially in a study like this with relatively few variables. For this reason, a second cluster analysis was calculated after slope aspect and previous stand composition were removed from the dataset.

A third analysis was performed in which slope aspect was transformed into a quantitative variable by applying a Heat Load Index (HLI) equation. Aspect categories North, East, South and West were assumed to be equal to 0, 90, 180 and 270 degrees respectively. Then angles were made into a linear scale, with the following equation:

$$HLI = [1 - \cos(angle - 45)] / 2$$

The underlying principle of this formula is that the southwest aspect (225 degrees east from north) receives the highest heat load, and any departure from this angle receives gradually less heat until reaching a minimum at the northeast slope (45 degrees east from north). The slope category "no aspect" was assumed to receive an intermediate heat load of 0.5. The transformation resulted in a variable that took three values: 0.15 for north and east slopes, 0.85 for south and west, and 0.5 for no aspect. This structure was assumed to reflect the simplicity of the previous indicator variables, but structured into only one variable.

All cluster tests had a small percent chaining, ranging from 1.26 to 1.87, which means almost no branching out of individual stands. Moreover, in all cases, clustering showed a consistent tendency to separate two equivalently large groups at first. Thus, for simplicity subsequent analyses were mostly devoted to study the characteristics of the two main groups of each test. Disease rating categories were then overlaid on each group of each cluster test (Figure 41).

As can be noticed by examining Table 23, the first cluster separated two groups that closely corresponded to N, W and NoA slope categories in one group, and E and S in the other. Whereas distribution of disease rates in the two groups (Figure 41) showed no trend.

The second cluster showed a reverse tendency: the first group had a predominance of higher disease rates and the second group has more stands with lower disease rates (Figure 41). Whereas slope frequencies appear more evenly distributed across the two cluster groups (slope frequencies were calculated after aspects were overlaid on the cluster results, since this variable was not used in the 2nd cluster analysis). Table 24 shows the distribution of variable values across groups in the cluster tests. Variables such as distance to the coast showed an important difference between groups in the second cluster analysis, in contrast to the first one, thus indicating that stands with lower disease rates tend to occur farther from the coast.

Results of the third test (with slope aspect made quantitative) resembled results of the first one, with a very uneven pattern of distribution of slope aspect categories across the two groups. Also consistently with the first test, disease categories were strikingly evenly distributed. This may indicate that the influence of slope aspect on the results was not obvious due to the fact that it was structured as four indicator variables.

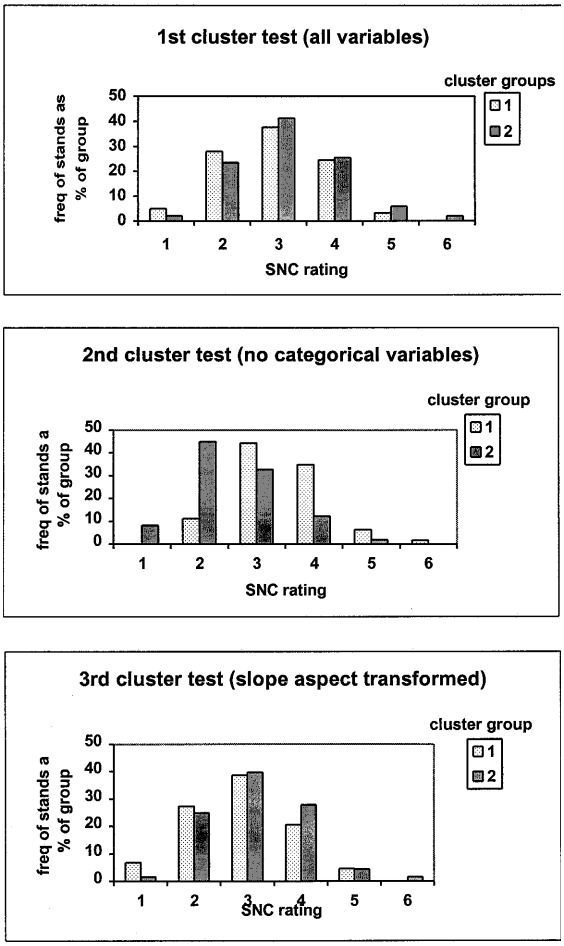

Figure 41. Resulting distribution of SNC rating categories after overlaid on cluster groups.

In synthesis, when slope aspect and previous stand composition were used, regardless of whether their structure was indicator variables or semi-quantitative, the stands grouped by clustering corresponded to slope aspect types. However, when only quantitative variables were used, the clustering separated stands with differences in SNC ratings. It should also be noted that with the 2nd cluster test, the group 1 of stands, has low frequencies of disease rates 1 and 2, and high frequencies of rates 3, 4 and 5 (Table 23). This confirms the idea suggested in Section V, that disease rates 1 and 2 can be grouped together as a "low disease"

Table 23. Distribution of stands by slope aspects between the 2 main groups of each cluster analysis

Cluster	Group	Frequencies of slope aspect (%)				
		N	E	S	W	NoA
1st	1	37.7	0.0	0.0	52.5	9.8
(all variables)	2	3.8	31.4	64.7	0.0	0.0
2nd	1	20.6	7.9	25.4	38.1	6.3
(no categorical variables)	2	22.4	22.4	34.7	16.3	4.2
3rd	1	56.8	34.1	0.0	0.0	9.1
(aspect transformed	2	0.0	1.5	48.5	47.1	2.9

Table 24. Average of stand environmental factors for groups in each cluster analysis

Cluster		1st (all variables)		2nd (no qualitative variables)		3rd (aspect transformed)	
Group		1	2	1	2	1	2
Elevation (ft)		694.5	788.9	559.5	966.3	625.0	810.3
Distance to the coast (mi)		8.44	8.40	5.27	12.48	7.96	8.72
Precipitation (mm)	Annual	2003.7	2171.2	1940.5	2259.3	1921.0	2182.8
	June	112.8	117.9	114.3	116.2	110.9	117.9
Max. temperature	Annual	152.7	151.1	153.7	149.7	153.2	151.2
(10th of °C)	June	186.6	183.5	182.1	189.2	185.6	184.9
Min. temperature	Annual	52.9	50.8	57.2	45.2	54.1	50.6
(10th of °C)	June	84.2	82.6	87.2	78.7	86.0	81.8

rate, and 3, 4, 5 and 6, can be considered "high disease" rate.

In conclusion, stand elevation, distance to the coast, precipitation and temperatures can separate two distinct groups of stands (see averages of 2nd cluster, Table 24) that also have different disease rating frequencies (2nd cluster, Figure 1), but not a clear slope aspect pattern (2nd cluster, Table 23).

VII. Relationship between disease and environment:

Non-metric multidimensional scaling procedure. Ordination techniques consist of summarizing the information contained in several variables by creating two or three axis along which samples units are placed for its analysis. Methods such as Non-metric Multidimensional Scaling (NMS) seem more appropriate than cluster analyses because NMS allows the quantification and graphical representation of the relationships among the environmental variables, and relationships between variables and an independent trait, such as the disease rating in the case of this study. Moreover, relationships are shown as a continuum, thus more accurately reflecting the real world. NMS seems an appropriate tool for cases in which parametric assumptions are difficult to fulfill. The method tries to minimize the "stress" of the configuration of the entities in the k-dimensional space ("k" is provided by the user). Stress is a measure of the lack of monotonicity between the original matrix dimension and the ordination space (Jongman et al. 1995).

In this study, it was decided to run the NMS ordination test two times; one with all the variables in the original form, and other with only the quantitative variables (slope aspect and previous stand composition excluded). As it was done with the cluster analysis, the disease information was overlaid on the results after the analysis was completed, to see if the SNC categories showed any pattern. The idea was to see whether or not this method would yield results equivalent to the 1st and 2nd cluster analyses. Because NMS is an iterative process in which the starting point, the number of dimensions of the final model and the number of iterations have to be specified, a preliminary test is carried out to help in the decision for the specifications in the definitive test. In this study, PC-ORD was used, and all tests started with a random seed number, 6 dimensions were specified for the final model to be achieved in 100 iterations. Also a Montecarlo test was included and set to run 20 times. After the preliminary run, each dimension was plotted vs. its value of stress, to determine at which minimum number of dimensions stress was substantially decreased. At that dimension, a graph of stress vs. number of iterations (provided in the output) was inspected to establish after what iteration stress did not significantly decrease (stabilization of stress reduction), and that number of iterations chosen as the definitive one. In all cases, Sorensen distance measure was used. Because the Sorensen method was going to be used for the first time in the study, a previous outlier analysis on Sorensen distances was done (other than distance method, the analysis was the same as the one in Section IV). Results were very similar to the ones in Section IV. Each preliminary run was done twice to ensure final stable solutions to choose from (McCune 1994).

In the first test (all the variables), a dimensionality of 3 was chosen and 60 iterations specified to do the definitive run. The definitive run of the second test (with no indicator variables) was done in 2 dimensions and 60 iterations. Final results are presented in Table 25.

The second test, with only quantitative variables, seemed to have yielded better results than the first one, as happened with the cluster analyses (1st and 2nd test, Section VI). Although both tests

had the same proportion of randomized runs with values equal or less than the stress in data ("p", Table 25) the second test resulted in a highly reduced stress only with 2 dimensions. Also the proportion of variance explained by the axes was higher in the second test.

For the purposes of this study the major interest was placed on the ordination results in terms of the distribution of disease severity. Figures 42, 43, and 44 show the ordination results with disease rates overlaid, for the axis 1 vs. 2, and axis 1 vs. 3 of the first test, and the axis 1 vs. 2 of the second test, respectively. In both of the first two graphs stands with different disease rates are completely intermingled. In contrast, the graph of the second test shows a promising trend, in which stands with rates 1 and 2 tend to occur towards the upper side of the graph, and 4 and 5 to the lower sides (dotted lines in Figure 44); rate 3 has the least clear pattern.

It is also interesting to note that the second test showed good correlation between the original variables and either one of the ordination axes. For example, both precipitation variables had an r-square of 0.6 - 0.80 with the first axis, and distance to the coast had 0.88 with axis 2. The minimum and maximum temperatures had a good correlation with axes 1 and 2 (0.5 - 0.7). In contrast, in the first test all variables had correlation of less than 0.59 in all axes. The higher correlation values with the ordination axes correspond to East aspect and non-Douglas-fir previous stand with axis 1; S, E and W aspects and Douglas-fir previous stand, with axis 2; and N and S aspects with axis 3. These results agree with the ones obtained in the cluster analyses: the ordination in first test was mostly influenced by slope aspect and previous stand composition.

In conclusion, stand elevation, distance to the coast, precipitation and temperatures can be reduced to two "variables" (ordination axes) that can discriminate to some degree stands with high and low SNC rating.

VIII. MRPP revisited

Since it was consistently found that the exclusion of aspect and previous stand yielded some degree of separation between stands with lower and higher disease ratings, it was decided to run a new MRPP only with the quantitative variables. Stands were grouped into five and two groups according to their disease ratings, as done in previous MRPP's. Then, the hypothesis of whether these groups are different in terms of their values of environmental variables, was tested.

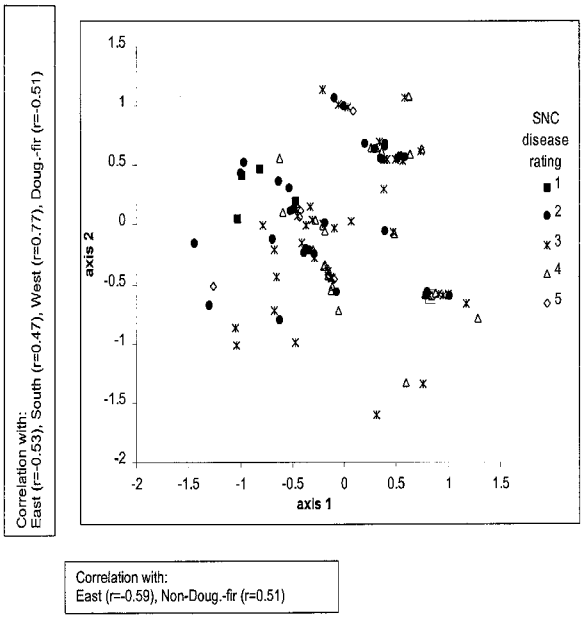

Figure 42. First NMS test, ordination axis 1 and 2.

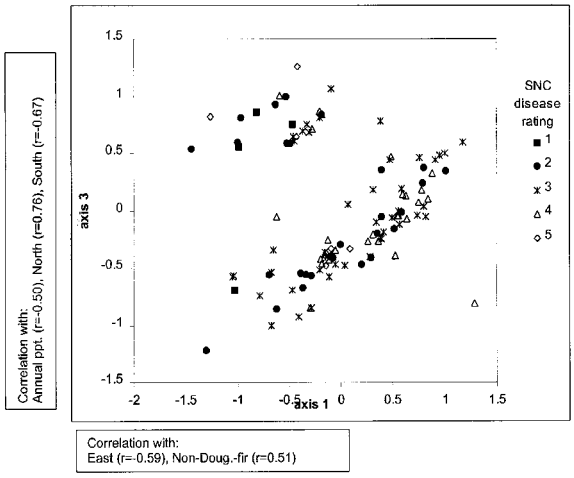

Figure 43. First NMS test, ordination axis 1 and 3.

Correlation with:

East (r=-0.59), Non-Doug.-fir (r=0.51)

The p-values support rejection of the null hypothesis that SNC rating groups are not different, for the five category as well as two category comparisons. R-values also are higher than previous MRPP's (Table 26), indicating that when slope aspect and previous stand composition were taken out of the analysis, the homogeneity within each group increased.

In conclusion, MRPP showed a statistically significant evidence that stands discriminated by disease ratings constitute also different groups in terms of elevation, distance to the coast, precipitation and temperature.

Table 25. NMS results

Test	Dimensions	Stress in data	Stress in Montecarlo	p	Cumulative % variance explained (1st, 2nd, 3rd axis)
1st (all variables)	3	14.04	18.40	0.05	35.5, 64.6, 86.8
2nd (qualitative variables)	2	12.32	21.97	0.05	61.1, 93.8, —

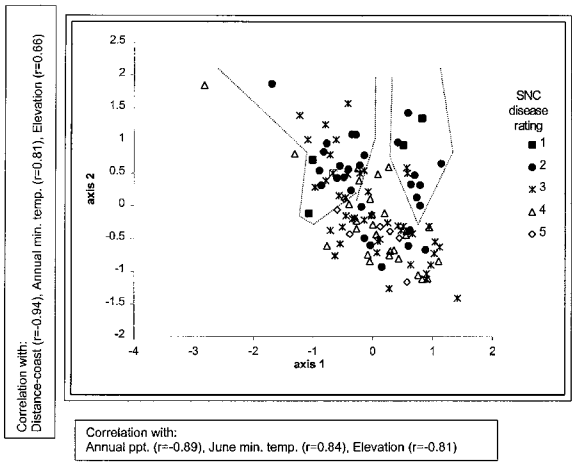

Figure 44 . Second NMS test, ordination axes 1 and 2.

IX. Discussion and conclusions

Based on the results of this study, it is concluded that a combination of some of the environmental variables used in this analysis have an evident association with occurrence and severity of Swiss needle cast disease. This is not a trivial finding, since very little is known about the factors that favor disease expression in general, and even less at the regional level. Most of the knowledge about this disease is based on circumstantial observation. This study points to the importance of climatic variables such as temperature; topographic characteristics such as altitude; and other site factors.

Slope aspect was included because it is reasonable to assume it has some influence on disease expression. This study, however, did not strongly support that idea. These results appear to indicate that disease severity (as expressed by the rating system) tends to be evenly distributed across different slope aspect categories. This relationship between the two attributes can be visualized in the "banded" distribution of the point markers in Figures 42 and 43. Similar conclusions can be made for the "previous stand composition" variable. It is also suggested that the results of the tests in relation to slope aspect (and probably also previous stand composition) do not depend on whether these variables are included as indicator or (semi-) quantitative variables. It seems that these variables strongly discriminate stands whereas the effect of the other more continuous variables is somehow relatively attenuated. This explanation, however, may only be applicable to the way the slope aspect was categorized in this study (i.e. five aspect categories, instead of nine or continuous). It is not known to what extent the use of a continuous slope aspect variable (such as heat load index transformation, Section VI) would change the outcomes of the tests. Nevertheless, the problem with categorical variables will always be present because not all variables can be made continuous.

Table 26. MRPP (with no categorical variables) results

	Group numbers and sizes	T (test)	p (prob.)	R
1st test (5 groups)	1=4, 2=29, 3=44, 4=28, 5=07	-5.24	0.0002	0.05
2nd test (2 groups)	1=33, 2=79	-10.10	0.0000	0.05

Distance to the coast is hard to interpret in epidemiological terms; it may be related with some kind of oceanic influence, such as moisture. This variable was included because its relationship with the disease has been empirically suggested.

The two main multivariate methods used to understand the relationship between environment and disease, cluster analysis and NMS yielded similar results, which will be used as a starting point for further research. Further trials are also being proposed to understand the validity of the disease rating system and its relationship to the MRPP test results.

This study was intended to be an exploratory analysis, for that reason less emphasis has been made on hypothesis testing, and more on visual and conceptual issues. It is suggested that future studies in this direction should: 1) make a thorough revision of the way in which variable values were assigned to the stands, 2) consider the inclusion of new variables and the exclusion of irrelevant or redundant variables (for example, temperatures of different months), 3) consider other ways of accounting for disease severity, and 4) try other multivariate methodologies in order to discriminate the effects of the methods from the effects of the "real world".

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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 forest land 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 reassert 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.