# Swiss Needle Cast

Research Cooperative annual report 2003

November 2003



OREGON STATE UNIVERSITY

# Members of the Swiss Needle Cast Cooperative and Their 2003 Contributions

Boise Building Solutions	\$22,500
Confederated Tribes of the Siletz	\$700
Hampton Resources, Inc.	\$22,500
John Hancock Life Insurance	\$22,500
Menasha Corporation	\$22,500
Oregon Department of Forestry	\$22,500
Simpson Timber Co.	\$22,500
Starker Forests	\$22,500
Plum Creek Timber Company	\$22,500
Weyerhaeuser Corporation	\$22,500
USDA Forest Service	In kind
USDI Bureau of Land Management	\$22,500
OSU Forest Research Laboratory (salary)	\$30,000

Cover: Map shows areas of Douglas-fir forests with symptoms of SNC detected during aerial survey in May 2003.

# Swiss Needle Cast Cooperative Annual Report

Edited by Greg Filip, SNCC Directo

2003



# **SNCC** income sources and expenditures 2003

Income	
Membership Dues	\$300,700
Oregon State Legislature	\$60,000
	(half-year)
Expenditures (as of 10/03)	
Salaries and Wages	\$173,400
OPE	60,100
Supplies and Services	99,800
Travel	8,500
Indirect Costs	24,500
Total Expenditures	\$366,300

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To: SNCC Members From: Greg Filip Date: September 2003 Subject: 2003 Annual Report

SNCC is now seven years old, and I thank the members for their support this year. For four years we were fortunate to receive \$480,000 from the Oregon State Legislature to support projects for 2000 to 2003. This year's annual report contains summaries on the progress made on our 8 projects. We had an aerial survey this year in Oregon that shows a slight decrease in Swiss needle cast. Information continues to be collected on the permanent growth impact and precommercial thinning plots. Progress continues on the basic infection biology research that is summarized in this report. Projects are continuing in tree physiology, soil and foliage nutrition, Bravo and sulfur application, and wood quality, and progress reports are contained in this report. Several publications concerning SNC were written this year based on results obtained through SNCC.

This year SNCC is at a crossroads. Our membership has voiced its opinion that SNCC may need to scale back and only conduct its long-term projects next year. In fact, there is a proposal to morph SNCC into a larger co-op that would deal with any forest pest problem should the need arise. Depending on the level of State Legislature support in 2004-05, the number of existing projects will probably be reduced in the future. With continued member, OSU, and State support, SNCC or a similar co-op hopes to continue to provide the quality research and extension as in the past.

I would like to especially thank this year's project investigators for their fine efforts in generating new information concerning Swiss needle cast: Alan Kanaskie, Doug Maguire, Katy Kavanagh, Jeff Stone, Dan Manter, Randy Johnson, Floyd Freeman, Robin Rose, Cathy Rose, Barb Gartner, and Gary Chastagner. And thanks to the many graduate students and research assistants who do so much of the work; Lori Winton, Julia Kerrigan, Paul Reeser, Doug Mainwaring, Aaron Weiskittel, and Wendy Sutton. 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, Charlie Moyer, Greg Johnson, Matt Higgins, Jim Carr, Mari Kramer, Will Littke, and Alan Kanaskie. We also produced a video on SNC this year to be shown at the World Forestry Congress in Quebec City this September. I would like to thank Mark Gourley, Walt Kastner, and Steve Hobbs for pulling this off on short notice.

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# **Highlights of 2003**

This report presents the Swiss Needle Cast Cooperative activities in Swiss needle cast research. Highlights for 2003 include:

- An aerial survey was conducted over 3 million acres in western Oregon. A total of 268,000 acres of Douglas-fir had obvious symptoms of Swiss needle cast. In general, symptoms of Swiss needle cast decreased in 2003 compared to 2002. Survey maps can be obtained from Alan Kanaskie, Oregon Department of Forestry in Salem (http:/ /www.odf.state.or.us/fa/FH/maps.htm).
- Research continues on 8 different projects in 2003 including: aerial and ground survey, growth impact studies, tree physiology, infection biology, precommercial thinning, nutrient imbalances, fungicide testing, and fertilizer and vegetation control.
- Aaron Weiskittel completed his MS thesis: "Alterations of Douglasfir crown structure, morphology, and dynamics imposed by the Swiss needle cast disease in the Oregon Coast Range."
- Randy Johnson published his research in the journal Silvae Genetica with the title "Genetic variation in tolerance of Douglas-fir to Swiss needle cast as assessed by symptom expression."
- Dan Manter, Lori Winton, Greg Filip, and Jeff Stone published their research in the Journal of Phytopathology as "Assessment of Swiss needle cast disease: temporal and spatial investigations of fungal colonization and symptom severity."
- Dan Manter, Barbara Bond, Katy Kavanagh, Jeff Stone, and Greg Filip published their research in the journal Ecological Modelling entitled "Modelling the impacts of the foliar pathogen, *Phaeocryptopus* gaeumannii, on Douglas-fir physiology: net canopy carbon assimilation, needle abscission and growth."
- Dan Manter published an additional paper in the Journal of Phytopathology called "Energy dissipation and photoinhibition in Douglas-fir needles with a fungal-mediated reduction in photosynthetic rates."
- Lori Winton, Dan Manter, Jeff Stone, and Everett Hansen published their research in Phytopathology entitled "Comparison of biochemical, molecular, and visual methods to quantify *Phaeocryptopus gaeumannii* in Douglas-fir foliage."

# Plans for 2004

- Continue aerial survey to monitor SNC in Oregon
- Continue to monitor plots in the Cascade foothills
- Monitor permanent plots from the growth impact study Phase III
- Conduct infection biology studies: Factors affecting colonization rate and foliage retention; *P. gaeumannii* infection, development,



and reproduction; aerobiology and epidemiology; and population biology of *P. gaeumannii* 

- Characterize the role of nitrogen in SNC; modifying foliar N to improve needle retention
- Determine impacts of SNC on wood quality of Douglas-fir
- Determine growth response to precommercial thinning in Douglasfir stands with varying intensity of SNC in the Coast Range of Oregon
- Continue to monitor the effects of fungicides, Bravo and sulfur, on SNC infection in coastal Douglas-fir
- Continue to monitor the effects of fertilization and vegetation control on SNC infection and growth of coastal Douglas-fir

# **Background and Organization**

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

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

#### Purpose

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

- 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 and nutritional biology, aerial and ground survey technology, growth and yield impacts, and strategies for control.
- 2 Conduct training and workshops on research and survey results
- 3. Provide newsletters and reports on research and surveys, and
- 4. Serve as a focal point for information on Swiss needle cast. Please visit our website at www.cof.orst.edu/coops/sncc/index.sht.



# Swiss Needle Cast Aerial Survey, 2003

Alan Kanaskie, ODF; Mike McWilliams, ODF; Keith Sprengel, USFS; and Dave Overhulser, ODF

#### Survey procedures:

The observation plane flew at 1,500 to 2,000 feet above the terrain, following north-south lines separated by 2 miles. Observers looked for areas of Douglas-fir forest with obvious yellow to yellow-brown foliage, a symptom of Swiss needle cast. Patches of forest with these symptoms (patches are referred to as polygons) were sketched onto computer touch screens displaying topographic maps or ortho-photos and the position of the aircraft.

Each polygon was classified for degree of discoloration as either "S" (severe) or "M" (moderate). Polygons classified as "S" for discoloration had very sparse crowns and brownish foliage, while those classified as "M" were predominantly yellow to yellow-brown foliage with slightly denser crowns than those classified as "S".

The Coast range was surveyed on May 19 to May 28, 2003. The area surveyed extended from the coastline eastward until obvious symptoms were no longer visible, and from the Columbia River south to Brookings.

We did not survey the Cascades in 2003, but Swiss needle cast does occur at damaging levels in some areas.

# **Results and discussion:**

Figure 1 shows the approximate size and location of areas of Coast range Douglas-fir forest with symptoms of Swiss needle cast detected during the survey conducted in May 2003. Figures 2,3, and 4 show the trend in damage from 1996 through 2003.

The 2003 Coast Range survey covered about 3 million acres of forest. Approximately 268,000 acres of Douglas-fir forest had obvious symptoms of Swiss needle cast; 196,000 north of the Lincoln-Lane county line, and 72,000 acres south of the Lincoln-Lane county line (figure 1). The easternmost area with obvious SNC symptoms was approximately 25 miles inland from the coast. Most areas with symptoms that could be detected from the air occurred within 18 miles of the coast. The trend in damage between 1996 and 2003 is shown in Table 1 and figures 2-11.

Some year-to-year variation in survey results is due to timing of the flights relative to the development of SNC symptoms. Because symptoms develop rapidly during April and May, later surveys usually detect more areas with symptoms than those conducted earlier. The 2003 survey was flown from May 19 to May 28, which is relatively late (the 2001 survey was flown from May 3 to May 10; the 2002 survey was flown from May 11 to May 31). This difference in timing of the survey, coupled with weather-driven tree phenological development, could account for much



of the yearly variation in survey results. Of possible significance is the fact that most acres mapped in 2002 and 2003 were classified by observers as "moderate", and very few acres were classified as "severe". Although this distinction in degrees of discoloration often is fickle, the difference between the 2002-2003 surveys and previous surveys is notable.

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



Figure 1. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in May, 2003.

Figure 2. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in May, 2002.

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, 2001.

 Table 1. Area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in 1996-2003.

Zone	1996	1997	1998	1999	2000	2001	2002	2003
Lincoln County & North	102,015	105,013	117,211	244,385	210,315	138,277	252,068	195,978
Lane County & South	29,073	39,089	54,916	49,264	71,888	74,188	134,972	71,87
Total	131,088	144,102	172,127	293,649	282,202	212,465	387,040	267,852

mates of disease impact on tree growth should not be made from the aerial survey alone. However, the aerial survey does provide a broad picture of the area significantly impacted by Swiss needle cast, which



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 2000.

Figure 5. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May, 1999.

Figure 6. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April and May, 1998 (does not include re-fly of Nehalem and Yamhill quadrangles).

from a practical point of view establishes a zone in which forest management should take into account the effects of the disease.

#### Acknowledgments:

The survey was conducted by the Oregon Department of Forestry Insect & Disease and Air Operations sections, and was funded by the Oregon State University Swiss Needle Cast Cooperative, the USDA Forest Service Forest Health Monitoring Program, and the Oregon Department of Forestry. Jim Baranek (ODF) piloted the plane. Mike McWilliams (ODF), Keith Sprengel (USFS), and Dave Overhulser (ODF) were the aerial observers.

#### Note:

The GIS data and a pdf file for the SNC survey can be accessed via the ODF web page at:

http://www.odf.state.or.us/fa/ FH/maps.htm



Figure 7. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April, 1997.

Figure 8. Areas of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during an aerial survey in April, 1996.



*Figure 9. Trend in area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in April and May, 1996-2003.* 



Figure 10. Area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in April and May, by zone, 1996-2003.



Figure 11. Area of Douglas-fir forest in western Oregon with symptoms of Swiss needle cast detected during aerial surveys in April and May, by county group, 1996-2003.

# *Trends in Damage from Swiss Needle Cast in Permanent Plots in 10- to 30-year-old Douglas-fir Plantations, November 2003*

Alan Kanaskie, ODF; Doug Maguire, OSU; Jon Laine, ODF; John Beeson, ODF; and Mike McWilliams, ODF

# Background:

The Permanent plot network was established in 1997 to provide a basis for monitoring Swiss needle cast (SNC) damage and for quantifying impacts of SNC on tree growth. This paper describes only the results of monitoring various indicators of SNC damage. Growth impacts are discussed in a separate paper (p 25).

# **Objectives:**

1) To describe the trends in the severity of damage from Swiss needle cast in randomly chosen 10- to 30-year-old Douglas-fir plantations in the Coast Range of western Oregon; 2) to estimate the area (acres) affected by SNC, and; 3) to ground-truth areas mapped by aerial survey.

# Methods

In 1997, 77 Douglas-fir plantations in the northern Coast Range of Oregon were randomly chosen for monitoring trends in damage from Swiss needle cast. The target population was all Douglas-fir plantations between 10 and 30 years total age (1996 age) and located within 18 miles of the coast, north of Newport and south of Astoria. With much cooperation from landowners, a list of plantations meeting these criteria was assembled. Plantations were selected from this list with probability proportional to size (area). The target population included 4,504 plantations covering 187,545 acres. The initial sample included 77 plantations covering 6,873 acres (figure 1). One plantation was lost from the study in 1999 due to cutting.

Swiss needle cast damage was assessed in April and May of each year since 1997. The 1997 assessments were based on a sample of ten trees per stand (two trees at each of five points along a transect). Beginning in 1998, assessments were made on ten trees per stand located in the 1/5acre permanent growth monitoring plots (Phase III) located at point 5 of the 1997 transects. The same ten trees in each plot were assessed each year unless mortality or breakage necessitated substitution.

#### **Stand Ratings**

Stand ratings were designed to provide a quick method of estimating Swiss needle cast severity by making a general assessment of average stand condition during a brief walkthrough of the stand. All ratings refer to the Douglas-fir component of the stand in the vicinity of the permanent plots. Overall stand discoloration was rated on a scale of 1 to 4 as follows:

1 = normal green color, with Douglas-fir similar in color to healthy hemlock; 2 = slight yellowing; 3 = moderate yellowing, and; 4 = severe yellowing and/or browning.

The Swiss Needle Cast Severity Rating for the stand was described according to the following 6-class system (needle retention was assessed on unshaded secondary laterals in the upper middle crown, usually whorl 5 to 7 from the tree top):

- 1 = Healthy, normal-appearing Douglas-fir stand. Typical of the eastslope Coast Range stands that are dark green, growing normally, and with normal needle retention (3.5 years or more midcrown). Douglas-fir and hemlock of the same size will not differ appreciably in color. Swiss needle cast may be present, but causing symptoms only on 3 year-old and older needles.
- 2 = Almost normal, but showing slight yellowing. Needle retention normal (3.5 or more years present on most trees) Douglas-fir will appear slightly more yellow than hemlock or spruce. Crown still appears full and dense. No reduction in height growth increment.
- 3 = Yellowing obvious. Most trees retaining 2.5 to 3 years of needles. No obvious height increment reduction.
- **4** = Yellowing obvious. Most trees retaining 1.5 to 2 years of

needles. Reduction in height growth increment by 25% of normal for one or more of the last three years will <u>not</u> be obvious, but may occur on a few trees only.

- 5 = Very yellow stand. Most trees retaining 1 to 1.5 years of needles. Height growth increment is reduced by at least 25% of normal for one or more of the last 3 years on at least 50% of trees, but not as much as described in "6" below.
- 6 =Stand is extremely yellow to yellow-brown, with very sparse foliage. Most trees retaining 1 year of needles or less in upper crown. Obvious height growth reduction for 4 or more years. These are the most severely damaged stands, typical of the Juno Hill, Beaver, and Hebo areas.

#### Individual tree Assessments on plots

Ten codominant or dominant trees in each 1/5-acre permanent plot were assessed for damage from Swiss needle cast. Sample trees were permanently tagged so the same trees could be assessed each year.

Needle Retention was estimated for the middle of each third of the live crown (upper, middle, lower) by examining secondary lateral branches and estimating the average number of annual needle compliments present (a secondary lateral is a branch that originates on the side of the main lateral branch). Sample branches were chosen to represent the average condition in the part of the crown being examined. The number of annual needle compliments present for each third of the live crown was estimated to the nearest 0.1 year as follows:

- **0.5** = 50 % of one-year-old needles (1998) remain, all older needles gone
- **1.0** = All one-year-old needles remain, older needles gone
- 1.2 = One-year-old needles plus 20% of two-year-old needles remain
- **1.6** = One-year-old needles plus 60% of two-year-old needles remain
- 2.0 = One- and two-year-old needles remain, older needles gone
- 2.5 = One- and two-year-old needles remain, plus 50% three year old needles remain
- **3.0** = All one-, two-, and three-year-old needles remain....

...and so on up to 6.0.

Whorl-5 needle retention was estimated by examining branches in the fifth whorl down (occasionally the sixth or seventh whorl) from the top of the tree. Needle retention, i.e., percentage of the full compliment of needles remaining on the branch at the time of the assessment, was estimated for each of the four most recent internodes of shoot growth on secondary laterals according to the following "0 to 9" scale:

- **0** = 0 to 10 percent of full compliment present;
- 1 = 11 to 20 percent of full compliment present;
- **2** = 21 to 30 percent of full compliment present;...
- **9** = 90 to 100 percent of full compliment present.

#### **Crown indicators**

From 1998 to 2000 inclusive, observers estimated the following four indicators, based in part on the US Forest Service Forest Health Monitoring protocols: 1)

*Crown Color* - discoloration of the upper 1/2 to 1/3 of crown, near whorls 5 to 7 from the top, using the 1 to 4 scale previously described for stand color; 2) *Crown Density* - estimate the percentage of sunlight being blocked by all parts of the crown, in 5% classes; 3) *Foliage Transparency* - estimate the percentage of sunlight being transmitted through the foliage, in 5% classes; 4) *Crown Dieback* - estimate the percentage of the total crown area that has dieback, in 5% classes. For the 2001 and subsequent assessments, crown



density and transparency variables were dropped because they had not proven very useful in previous analyses.

#### **Results and Discussion**

The mean SNC stand rating (1 to 6 scale) increased gradually by nearly one rating class between 1997 and 2003 (figure 2). This trend suggests a general increase in SNC damage over the period based on subjective overview ratings. Although the SNC rating for 2003 was not significantly different from the 2002 rating, the ratings for these two years were significantly greater than in previous years.

In contrast, the mean stand discoloration rating did not differ consistently during the same period, suggesting a trend of slightly improving stand color between 1997 and 2001, then an increase in 2002 and 2003 to 1997 levels (figure 3). One explana-



Figure 2. Mean SNC Stand Rating for 76 permanent plots in 10- to 30-year old (1996 age) Douglas-fir plantations. Swiss needle cast severity increases as SNC Stand rating increases. Means with same letter within a data series are not significantly different (analysis of variance, Fisher's LSD, a=.05).



Figure 1. Location of 76 permanent plots for monitoring Swiss needle cast and tree growth in 10- to 30-year-old Douglas-fir plantations, Coast Range, Oregon.

Figure 3. Mean SNC Stand Discoloration Rating for 76 permanent plots in 10- to 30-year old (1996 age) Douglas-fir plantations. Stands become more discolored (more yellow) as Stand Discoloration Rating increases. Means with same letter within a data series are not significantly different (analysis of variance, Fisher's LSD, a=.05).

tion for the discrepancy between these two stand ratings is that the SNC rating incorporates both needle retention and color into the rating. A stand that is very yellow but with good needle retention could lead to different relative ratings on each scale. The SNC rating (1-6) is the preferred method for overview rating stands because it incorporates many indicators of Swiss needle cast damage. The stand color rating was originally conceived as a link to aerial survey and remote sensing applications. In practice, the stand discoloration rating has proven very difficult to determine with consistency because of the influence of sunlight, cloud cover, and observer subjectivity. The SNC rating often is not consistent with needle retention ratings, largely because the SNC rating tends to focus on condition of the upper crown, which is the only part visible from outside of the stand.

Mean needle retention (whole crown) for all plots increased slightly from 1997 to 1999, decreased from 1999 to 2001, then increased gradually to the highest level (2.54 annual needle compliments) since measurements began. Although the differences were significant (Analysis of variance, .05 significance level), they were very small (figure 4).

Analysis by crown thirds consistently has shown that mean needle retention is lowest in the upper third of the tree crown and greatest in the lower third of the tree crown. Mean needle retention in the upper third of the crown showed a noticeable but slight decrease from 1998 to 1999, probably reflecting the interaction of Swiss needle cast with the high fre-



Figure 4. Mean needle retention for 76 permanent plots in 10- to 30-year old (1996 age) Douglas-fir plantations. Means with same letter are not significantly different (analysis of variance, Fisher's LSD, a=.05).

quency of severe windstorms and a period of very cold weather that occurred during the winter of 1998-1999 (Figure 5). Slight improvements in needle retention in 2001 - 2003 could be due to recent relatively mild winters, as well as increasing tree size and subsequent crown sheltering, both of which could reduce foliage loss.

Mean needle retention for each permanent plot appears in figure 6. Mean needle retention (whole crown) differed significantly (analysis of variance, paired t-tests, a=.05) between 1998 and 2003 on 13 (17 percent) of the plots. During this period, mean needle retention increased on 4 of the plots, and decreased on 9 of the plots (figure 7). We chose 1998 as the reference year rather than 1997 because the 1997 data were from transect trees, while all subsequent data were from permanent plot trees, with the same trees being measured each year. The largest improvement in needle retention for an individual stand during this period was 0.71 annual needle compliments; the largest decrease in retention was 1.14 annual needle compliments. We did not observe any geographic pattern



*Figure 5. Mean needle retention for 76 permanent plots in 10- to 30-year-old (1996 age) Douglas-fir plantations, by crown thirds.* 

to the changes in needle retention.

Mean needle retention ratings were expanded to estimate the number of acres in each needle retention class for the 187,545-acre population. Since 1997, there has been a general increase in the estimated number of



#### Stand Number

Figure 6. Mean needle retention (whole crown) in May 2003 for each of the 76 permanent plots in 10- to 30-year-old (1996 age) Douglas-fir plantations.

acres with needle retention of at least 2.25 annual compliments, and a general decrease in estimated acres with less than 2.25 annual compliments (figure 8).

Retention

Foliage

Mean

#### Conclusions

Based primarily on needle retention ratings, these results show little evidence of a significant change in damage from Swiss needle cast since 1997. The slight but significant increase in mean needle retention from 2001 to 2002, and the lack of a consistent trend of worsening damage from SNC are encouraging, and are consistent with casual observations in the north coast area. However, the overall poor needle retention in the sample population suggests a continuing severe growth reduction from Swiss needle cast.



Figure 7. Increase or decrease in mean needle retention between 1998 and 2003 in 76 permanent monitoring plots in 10- to 30-year-old (1996 age) Douglas-fir plantations. Mean needle retention increased significantly on 4 of the plots, and decreased significantly on 9 of the plots (analysis of variance, Fisher's LSD a = 0.05).



Figure 8. Distribution of Douglas-fir plantation acreage by needle retention class for the 187,545-acre population from which sample plantations were chosen.

# Precommercial Thinning of Douglas-fir Stands with Varying Intensity of Swiss Needle Cast in the Coast Range of Oregon: Status Report on Trends in Disease Severity, November 2003.

Alan Kanaskie, ODF; Doug Maguire, OSU; Mike McWilliams, Jon Laine, and John Beeson, ODF.

# Background

Many young Douglas-fir plantations in coastal Oregon exhibit extreme symptoms of Swiss needle cast, and these symptoms are associated with reduction in tree growth. Observations suggest that thinning stands with severe Swiss needle cast may increase foliage loss and discoloration, 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 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. This reports focuses on trends in the various indices of disease severity between 1998 and 2003. Tree growth responses to pre-commercial thinning and Swiss needle cast appear in a separate report.

# Methods

In April and May of 1998, twenty-three paired 0.2 acre square plots were installed in 10- to 16-year-old Douglas-fir plantations (1997 age) in northwest Oregon. Plot locations were selected across a range of Swiss needle cast severity classes and distributed across different topographic aspects (figure 1). One plot in each pair was precommercially thinned to approximately 200 trees per acre in May 1998 (because of initial stocking levels, at two sites the target residual was 100 trees per acre). At five of the 23 locations, an additional plot was thinned to approximately 100 trees per acre. During thinning, tree spacing was given priority over tree



quality. All crop trees were measured for dbh, total height, and height to crown. Swiss needle cast severity (needle retention and discoloration) was assessed annually during April and May each year since plot establishment. Growth measurements are taken every two years.

#### **Stand Ratings**



Figure 1. Location of 23 permanent plot sets to monitor disease symptoms and evaluate growth response to pre-commercial thinning in Douglas-fir plantations with varying intensity of Swiss needle cast in the Coast range of Oregon.

Stand ratings were designed to provide a quick method of estimating Swiss needle cast severity by making a general assessment of average stand condition during a brief walk-through of the stand. All ratings refer to the Douglas-fir component of the stand in the vicinity of the permanent plots. Overall stand discoloration was rated on a scale of 1 to 4 for as follows:

- 1 = normal green color, with Douglasfir similar in color to healthy hemlock;
  2 = slight yellowing;
  3 = moderate yellowing, and;
  4 = severe yellowing and/or browning.
- The Swiss Needle Cast Severity Rating for the stand was described according to the following 6class system (needle retention was assessed on unshaded secondary laterals in the upper middle crown, usually whorl 5 to 7 from the tree top):
- 1 = Healthy, normal-appearing Douglas-fir stand. Typical of the eastslope Coast Range stands that are dark green, growing normally, and with normal needle retention (3.5 years or more midcrown). Douglas-fir and hemlock of the same size will not differ appreciably in color. Swiss needle cast may be present, but causing symptoms only on 3 year-old and older needles.
- 2 = Almost normal, but showing slight yellowing. Needle retention normal (3.5 or more years present on most trees) Douglas-fir will appear slightly more yellow than hemlock or spruce. Crown still appears full and dense. No reduction in height growth increment.
- **3** = *Yellowing obvious.* Most trees retaining 2.5 to 3 years of needles. No obvious height increment reduction.
- 4 = Yellowing obvious. Most trees retaining 1.5 to 2 years of

needles. Reduction in height growth increment by 25% of normal for one or more of the last three years will <u>not</u> be obvious, but may occur on a few trees only.

- 5 = Very yellow stand. Most trees retaining 1 to 1.5 years of needles. Height growth increment is reduced by at least 25 percent of normal for one or more of the last 3 years on at least 50% of trees, but not as much as described in "6" below.
- 6 = Stand is extremely yellow to yellow-brown, with very sparse foliage. Most trees retaining 1 year of needles or less in upper crown. Obvious height growth reduction for 4 or more years. These are the most severely damaged stands, typical of the Juno Hill, Beaver, and Hebo areas.

#### Individual tree Assessments on plots

Ten codominant or dominant trees in each 1/5-acre permanent plot were assessed for damage from Swiss needle cast. Sample trees were permanently tagged so the same trees could be assessed each year.

Needle Retention was estimated for the middle of each third of the live crown (upper, middle, lower) by examining secondary lateral branches and estimating the average number of annual needle compliments present (a *secondary lateral* is a branch that originates on the side of the main lateral branch). Sample branches were chosen to represent the average condition in the part of the crown being examined. The number of annual needle compliments present for each third of the live crown was estimated to the nearest 0.1 year as follows:

- **0.5** = 50 % of one-year-old needles (1998) remain, all older needles gone
- **1.0** = All one-year-old needles remain, older needles gone
- 1.2 = One-year-old needles plus 20% of two-year-old needles remain
- **1.6** = One-year-old needles plus 60 % of two-year-old needles remain
- 2.0 = One- and two-year-old needles remain, older needles gone
- 2.5 = One- and two-year-old needles remain, plus 50% three year old needles remain
- **3.0** = All one-, two-, and three-yearold needles remain....

...and so on up to 6.0.

Whorl-5 needle retention was estimated by examining branches in the fifth whorl (occasionally the sixth or seventh whorl) from the top of the tree. Needle retention, i.e., percentage of the full compliment of needles remaining on the branch at the time of the assessment, was estimated for each of the four most recent internodes of shoot growth on secondary laterals according to the following "0 to 9" scale:

- **0** = 0 to 10 percent of full compliment present;
- 1 = 11 to 20 percent of full compliment present;
- **2** = 21 to 30 percent of full compliment present;...
- **9** = 90 to 100 percent of full compliment present.

#### **Crown indicators**

From 1998 to 2000 inclusive, observers estimated the following four indicators, based in part on the US Forest Service Forest Health Monitoring protocols: 1)

Crown Color - discoloration of the upper 1/2 to 1/3 of crown, near whorls 5 to 7 from the top, using the 1 to 4 scale previously described for stand color; 2) Crown Density - estimate the percentage of sunlight being blocked by all parts of the crown, in 5% classes; 3) Foliage Transparency - estimate the percentage of sunlight being transmitted through the foliage, in 5% classes; 4) Crown Dieback - estimate the percentage of the total crown area that has dieback, in 5% classes. For the 2001 and For the 2001 and subsequent assessments, the crown density and transparency variables were dropped because they had not proven very useful in previous analyses.

#### **Results and Discussion**

Analysis of data for all 23 sites revealed few trends. Four growing seasons after thinning, mean needle retention did not differ significantly between thinned and unthinned plots (analysis of variance, Fisher's LSD,  $\alpha$ =05). Mean needle retention also did not differ significantly among the five annual measurements for any of the treatments (analysis of variance, 0.05 significance level) (figure 2). There was no significant difference in Stand Swiss Needle Cast Severity or Discoloration Ratings among years or between thinning treatments (figure 3).

Analysis of data from each installation separately revealed that mean needle retention in 2002 differed significantly between thinned and unthinned plots at 7 of the 23 sites (T100 and T200 treatments were combined for this analysis). At six of these sites (APT4, APT5, APT6, Nataxe, Axe,



Figure 2. Mean needle retention (whole-crown) in paired thinned and unthinned plots in Douglas-fir plantations affected by Swiss needle cast in the Coast Range of northwest Oregon, 1998 to 2003. Plots were thinned in May 1998. Needle retention was evaluated in May of each year. Mean needle retention did not differ significantly (analysis of variance,  $\alpha$ =.05) between thinned and unthinned plots, or among years. Steere, and Steinburg), trees in the thinned plots had greater needle retention than trees in the unthinned plots. At the other site (Powerline - Tillamook County), mean needle retention of trees in the unthinned plot was greater than needle retention trees in the thinned plot (analysis of variance, 0.05 significance level). The magnitude of difference in needle retention between thinned and unthinned plots on these seven sites ranged from 0.5 to 1.0 annual needle compliments (figure 4).

Five of the sites received two levels of thinning; 100(T100) and 200 (T200) residual trees per acre. A comparison of mean mid-crown needle retention among treatments at these sites five growing seasons after thinning showed significant differences among thinning treatments at three of the five sites (figure 5). At two sites, APT5 and APT6, mean needle retention was greater in the thinned plots than the unthinned plots, but did not differ between the T100 and T200 treatments. At the other site (Devitt), mean needle retention in the T100 plot was significantly less than in the T200 plot and the unthinned plot.

Casual observations have lead to speculation that Douglas-fir plantations with severe Swiss needle cast and poor needle retention will experience more needle loss following precommercial thinning than unthinned plantations. Analysis of the six stands with the lowest needle retention at the time of thinning and the six stands with the greatest needle retention at the time of thinning showed little difference in the effects of thinning on needle retention four



Figure 3. Swiss Needle Cast Stand Severity Rating for paired thinned and unthinned plots in Douglas-fir plantations affected by Swiss needle cast in the Coast Range of northwest Oregon, 1998 to 2003. Plots were thinned in May 1998. The SNC severity rating did not differ significantly (analysis of variance,  $\alpha = 0.05$ ) between thinned and unthinned plots, or among years.



Figure 4. Mean difference in mid-crown needle retention in 2003 for 23 paired thinned and unthinned plots of Douglas-fir affected by Swiss needle cast in the Coast Range of northwest Oregon. Plots were thinned in May 1998. Needle retention was evaluated in May of each year. A vertical bar that extends below the zero line indicates that needle retention in the thinned plot was less than in the unthinned plot; if above the line, needle retention in the thinned plot was greater than in the unthinned plot. Significant differences are indicated by "s" (t-test,  $\alpha = 0.05$ ). T100 and T200 treatments were combined for this analysis.

Figure 5. Mean needle retention (mid-crown) in 2003 for sites with three treatments (not thinned; T100 = 100trees per acre after thinning; T200 = 200 trees per acre after thinning). Means within a site with the same letter are not significantly different (Analysis of variance, Fisher's LSD,  $\alpha = 0.05$ ).



growing seasons after thinning. Needle retention was significantly lower in the thinned plot at only one of the six sites with the poorest initial needle retention (Figure 6), and at none of the six sites with the highest initial needle retention (Figure 7).

Needle retention is not the only measure of the effects of thinning on tree damaged by Swiss needle cast. A small amount of tree fall, top breakage, and branch dieback occurred at low levels in a few of the thinned plots, but not in the unthinned plots, and especially in plantations with the most severe Swiss needle cast.

A trend appears to be developing for improved needle retention in thinned plots. The expected high loss of foliage following precommercial thinning of stands damaged by Swiss needle cast has not occurred five growing seasons after thinning. Even sites with severe disease (such as



Figure 6. Mean needle retention (mid-crown) in 2003 for paired thinned and unthinned plots with the lowest initial (1998) needle retention. An arrow indicates sites with a significant difference in mean needle retention between thinned and unthinned plots (t-test,  $\alpha$ =0.05).



Figure 7. Mean needle retention (mid-crown) in 2003 for paired thinned and unthinned plots of Douglas-fir with the greatest initial (1998) needle retention. An arrow indicates the sites with a significant difference in mean needle retention between thinned and unthinned plots (t-test,  $\alpha$ =05).

Juno Hill and Beaver) showed little difference in mean needle retention between thinned and unthinned plots. Needle retention ratings, although correlated with tree volume growth, likely do not capture the entire the impact of the Swiss needle cast on tree growth. Retention ratings do not account for shoot length, needle size and quality, crown length, or the absolute amount of foliage present, all of which vary considerably in stands affected by Swiss needle cast. A differential tree growth response to thinning across a range of Swiss needle cast damage still is quite possible, despite the inconclusive needle retention results.

The stands in this study were approximate 12 to 16 years old at the time of thinning. Trees of this age typically have deep crowns and are growing vigorously. Older overstocked stands with relatively low crown ratios could respond quite differently to thinning than the stands in this study.

#### Conclusions

These results suggest that precommercial thinning does not have an obvious detrimental effect on Douglas-fir plantations affected by Swiss needle cast in the Coast range of Oregon. The 2003 data suggest a slight improvement in needle retention in the thinned plots. Precommercial thinning remains a viable stand management tool in the Coast Range in all but the most severely damaged stands.

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# *Trends in Symptom Severity after Precommercial Thinning in Douglas-fir Stands with Differing Initial Swiss Needle Cast Severity*

Doug Maguire, OSU; Alan Kanaskie, ODF; and Doug Mainwaring, OSU

#### Abstract

A study of paired plots, one thinned and one control, was established in 1998. Plots have received Swiss needle cast ratings for 6 consecutive years now. Statistical analysis indicates that a treatment effect is now starting to appear. Foliage retention on trees from thinned plots has become significantly greater than on trees from control plots. All 22 sets of plots will be remeasured this year to allow growth assessment after three 2-yr growth periods.

## Introduction

Concern remains about the effect of thinning on Swiss needle cast (SNC) intensification. Some anecdotal evidence suggests that thinning will not eliminate or ameliorate the disease, but rather intensify the symptoms. In some areas, commercially thinned stands with few initial SNC symptoms have been clearcut several years after thinning due to their apparently rapid decline and intensification of SNC. In addition, the lack of thinning response in stands with moderate SNC raises the question as to whether stand management objectives normally attainable by thinning can be met under moderate or severe SNC. The objective of this report is to provide an updated assessment of thinning effects on SNC symptoms. All plots will be measured during the 2003-04 dormant season, after which an update on growth responses after three 2-yr growth periods will also be available.

# Methods

In the late winter/early spring of 1998, 22 sets of plots were established across a range in initial Swiss needle cast (SNC) severity. Most of these sets contained a pair of plots, one thinned and the other a control, but some included a third plot that allowed testing of two different residual densities. The thinning prescription specifically called for leaving 494 tph (200 tpa), but on two installations the target residual was 247 tph (100 tph) because stand densities were already low. In addition, a third plot was established on 5 installations to yield two thinned plots, one with a residual of 494 tph and one with a residual of 247 tph. All control plots and 494-tph plots were square and covered 0.08-ha (1/5-ac; 31.8 x 31.8 m), except for the two installations on which the thinned plot was reduced to 247-tph. These latter two control plots and all 7 plots thinned to 247 tph encompassed an area of 0.16-ha (2/5-ac). On each measurement plot, all trees were tagged and measured for diameter at breast height. Ten dominant or codominant trees on each plot were also scored for SNC at time of plot establishment in 1998 and during annual visits in the spring (1999-2003).

An analysis of variance with repeated measures was performed to test whether the thinning treatments caused any downward or upward trend in SNC, and indicated by foliage retention.

#### **Results and Discussion**

Needle retention initially was quite similar between control plots and those thinned to 200 tpa. The five plots thinned to 100 tpa had slightly greater needle retention to begin with, due to random assignment of treatments at a site. However, significant time by treatment interactions underscore the pattern in improved needle retention that seems to characterize the thinned plots. This treatment effect emerged only in the last year or two, and is particularly apparent in the middle and lower part of the crown (Figs. 1-4). Remeasurements performed during the current (2003-04) dormant season will reveal whether there is a corresponding growth improvement as well.



Figure 1. Average needle retention across crown thirds in the spring of each year since plot establishment. Plots were thinned in early 1998.



Figure 2. Average needle retention in upper crown third in the spring of each year since plot establishment. Plots were thinned in early 1998.



Figure 3. Average needle retention in middle crown third in the spring of each year since plot establishment. Plots were thinned in early 1998.



Figure 4. Average needle retention in lower crown third in the spring of each year since plot establishment. Plots were thinned in early 1998.

# *Growth Impact Study: Trends in Swiss Needle Cast Symptoms Over Six Years of Annual Severity Ratings*

Doug Maguire, OSU; Alan Kanaskie, ODF; Doug Mainwaring, OSU; Randy Johnson, USDA-FS PNW Research Station; and Greg Johnson, Weyerhaeuser

#### Abstract

Permanent plots in the Growth Impact Study have been rated annually for Swiss needle cast severity from 1998 through 2003. Although changes in ratings have occurred from year to year, there is no consistent downward or upward trend for individual plots. As a result, initial rankings in SNC severity have remained generally stable; the stands in poorest condition in 1998 continue to exhibit the most severe symptoms in 2003.

#### Introduction

The Growth Impact Study (GIS) was initiated in 1997 to address two major objectives: 1) to monitor Swiss needle cast (SNC) symptoms and tree growth in 10-30-yr-old Douglas-fir plantations in north coastal Oregon; and 2) to provide an improved estimate of growth losses associated with a given initial level of SNC. Retrospective work conducted in the spring of 1997 established growth losses across a range in SNC severity (Maguire et al. 1998, 2002). Volume growth losses were estimated to average 23% for the target population in 1996, with losses reaching almost 50% in the most severely impacted stands. Permanent plots established in the spring of 1998 and remeasured in 2000 and 2002 confirmed these growth losses. Remeasurement for a third 2-yr growth period will be completed during the dormant season of 2003-04. This report updates the trend in SNC symptoms through the spring of 2003.

# Methods

In the late winter/early spring of 1998, a network of 76 permanent plots was established at locations previously sampled in Phases I and II (retrospective phase) of the Growth Impact Study. The plots were square and 0.08-ha (1/5-ac) in area (31.8 x 31.8 m). Each plot was centered on the 5th point of the ODF transect established in Spring 1997 (Phase I plots were centered on the 3rd point). Trees on each plot were scored for SNC at time of plot establishment in 1998 and just prior to bud break each year from 1999 through 2003. On 10 dominant or codominant trees per plot, the crown was divided vertically into thirds, and the average number of





years that foliage was retained in each third was estimated visually to the nearest 0.1 year. Plot ratings were computed as the average of all crown thirds from all ten trees. In addition to this standard foliage retention rating, the proportion of foliage surviving in age classes 1 to 4 yrs was recorded, as was crown color.

#### Data analysis

Average ratings for each plot were computed by averaging the three ratings for each tree and then averaging the ten trees rated per plot. Rank correlations in SNC severity were performed among the six years of monitoring, and the consistency in relative SNC severity was assessed graphically.

#### **Results and Discussion**

As reported last year (2002), extremes in foliage retention have generally moved toward more moderate values since 1997; that is, stands with severe SNC in 1997 have had an improvement in foliage retention and stands with few SNC symptoms have experienced a slight decline in foliage retention (Figs. 1 and 2).

The correlation between foliage retention in 1998 and subsequent years declines with increasing time (Fig. 3). This decline in correlation holds true regardless of the year established as baseline (Fig. 4). The change in foliage retention does not seem to be consistent for plots; that is, they tend to fluctuate up and down, rather than having a consistent trend in one direction. Likewise, the direction and degree of change in foliage retention is not conditional on initial Swiss needle cast severity (Fig. 5). In general, the stands exhibiting the most severe Swiss needle cast continue to be the worst stands, and those that are most healthy continue to show relatively few symptoms.



Figure 1. Foliage retention for each plot in 1998 and 2003 plotted in order of retention in 1998.



Figure 2. Foliage retention for each plot in 2003 plotted again retention in 1998. The 1:1 line represents no change from initial foliage retention in 1998.



Figure 3. Correlation between base year foliage retention and foliage retention in subsequent years.



Figure 4. Correlation between base year foliage retention and foliage retention in subsequent years plotted as years since initial foliage retention level.



Figure 5. Change in foliage retention from 1998 to 2003 plotted against initial foliage retention in 1998. Positive values represent improvements in foliage retention and negative values represent declines in foliage retention.

#### **Literature Cited**

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# Impact of Swiss Needle Cast on Wood Quality of Douglas-fir

Barb Gartner, OSU; Randy Johnson, USDA-FS; Amy Grotta, OSU; Doug Maguire, OSU; and Alan Kanaskie, ODF

## Introduction

This study investigated how wood properties such as strength and stiffness are affected by SNC. An earlier experiment in the Bravo-sprayed plots (Johnson et al. 2003) found that diseased trees had higher wood density due to increased latewood proportion. In the current study, we expanded the scope of wood properties examined to include modulus of elasticity (MOE, a measure of stiffness), modulus of rupture (MOR, a measure of strength), and microfibril angle (MFA). In contrast to the Bravo experiment, we looked at trees in stands with varying degrees of severity of SNC. A description of our sampling strategy and some early results were reported at last year's annual meeting (Gartner et al. 2002). Since then we have completed the testing and analysis of all samples and are currently refining the data analysis. Here we present a synopsis of our findings.

#### Methods

A full description of site and tree selection is detailed in Gartner et al. (2002). Briefly, 15 stands were selected from the existing set of Swiss needle cast Growth Impact Plots on the western slope of the Coast Range. Stands were selected so that they fell within a relatively narrow band of site index and stand age, but with a wide range needle retention values recorded in 2001. In each stand, 12 dominant to co-dominant trees were felled in spring 2002. From each tree, a 30 cm section of an internode at approximately breast height was cut, along with a 5 cm disk at breast height and another at the crown base. In addition to the stand level needle retention values, needle retention was assessed for three of the felled trees selected at random from each stand, which allowed us to examine some tree-level associations.

From each of the 30 cm sections, eight vertical beams (each 1 cm wide tangentially, 1 cm deep and at least 16 cm long) were cut from around the perimeter of the disk so that they contained the outermost growth rings. The beams were subjected to static bending tests on an Instron<sup>TM</sup> universal testing machine according to ASTM standard D 143-94 (ASTM 1999) with minor modifications. The beam supports were set 15 cm apart for a 15:1 span:depth ratio, and the beams were placed so that the load was applied to the tangential face closest to the pith. The load was applied continuously at a rate of 5 mm/min. MOE and MOR

were then calculated for each beam according to the formulas specified in Markwardt and Wilson (1935). For each beam, we determined its density at 12% moisture content and counted the number of annual rings present on the 1 cm x 1 cm crosssectional face. We also recorded whether earlywood, latewood, or transition wood was present on the top and bottom faces of the beam (relative to the load); although these data have not yet been included in our analysis, in our final analysis they will be incorporated in our model in order to reduce within-tree variation.

From the breast height disk, a diametric strip 1.5 cm wide tangentially and 1.5 cm tall was sawn. The location of the sample was selected so that it was neither the longest nor shortest diameter and consisted of clear wood. From one end of this strip (chosen at random), the outermost 2 cm of radial growth was cut and sent to the SilviScan facility at CSIRO in Australia. SilviScan is an automated wood analysis tool which quickly measures wood density and estimates MFA through x-ray diffraction (Evans and Ilic 2001). These measures are then used to estimate MOE. Density was measured at 0.1 mm intervals along the cross-section. For trees with >5 rings/cm, MFA was measured at 0.2 mm intervals, whereas for trees with  $\leq 5$  rings/cm, MFA was measured at 0.5 mm intervals. For each tree, SilviScan provided overall and ring-by-ring values for ring width, earlywood width, latewood width, latewood proportion, ring density, earlywood density, latewood density, earlywood MFA, latewood MFA, and estimated MOE.

#### **Data Analysis and Results**

MOE, MOR, and rings/cm for each of the 8 beams per tree were averaged to obtain values for each tree. Stand level averages for these variables along with breast height age, DBH, and all of the data from SilviScan were obtained by averaging the values from each of the 12 trees per stand (Table 1). All variables showed significant differences among stands with associated p-values < 0.05. The distribution of variance components for among-stands and within-stand are shown in Table 1. The proportion of variance was high among stands (and consequently, low within stands) for needle retention, rings/cm, latewood density, and latewood proportion. The proportion of variance was low among stands (and consequently, high within stands) for DBH, ring width, MOE, MOR, earlywood density, and both earlywood and latewood MFA.

At the among-stand level, we used correlation (r) and simple linear regression to examine the association between the 2001 stand needle retention score and each wood property. The association of needle retention with wood properties at the treewithin-stand level was examined using random coefficient modeling using the SAS Mixed procedure (Littell et al. 1996). Random coefficient modeling assumes that the regression model within each stand (for individual tree needle retention and a wood property) is a random sample from the population of all stands; and that each regression model is a random deviation from some population regression model. The analysis then uses the regression models from all the sampled stands to derive an estimate of the "population" regression model; a model that would describe the general within-

Table 1. Stand-level summary data and proportion of the variation attributed to the among-stand and withinstand variance components (n=15)

				Variance component	
Stand Mean	Std. Dev.	Min	Μαχ	Among stands	Within stand
2.3	0.4	1.7	2.9	58%	42%
26.7	2.7	21.3	31.0	18%	<b>82</b> %
4.4	1.3	2.5	7.0	55%	45%
2.5	0.8	1.3	4.4	38%	<b>62</b> %
10,394	781	9,333	11,698	18%	82%
99.9	6.8	88.9	112.9	20%	80%
0.60	0.04	0.52	0.68	32%	<b>68</b> %
0.31	0.02	0.28	0.35	11%	<b>89</b> %
0.83	0.05	0.74	0.92	<b>46</b> %	54%
0.53	0.05	0.45	0.61	<b>29</b> %	71%
17.9	1.6	14.9	21.1	10%	<b>90</b> %
11.9	1.1	9.6	14.1	10%	<b>90</b> %
	Stand Mean 2.3 26.7 4.4 2.5 10,394 99.9 0.60 0.31 0.83 0.53 17.9 11.9	Stand Mean         Std. Dev.           2.3         0.4           26.7         2.7           4.4         1.3           2.5         0.8           10,394         781           99.9         6.8           0.60         0.04           0.31         0.02           0.83         0.05           17.9         1.6           11.9         1.1	Stand Mean         Std. Dev.         Min           2.3         0.4         1.7           26.7         2.7         21.3           4.4         1.3         2.5           2.5         0.8         1.3           10,394         781         9,333           99.9         6.8         88.9           0.60         0.04         0.52           0.31         0.02         0.28           0.83         0.05         0.45           17.9         1.6         14.9           11.9         1.1         9.6	Stand Mean         Std. Dev.         Min         Max           2.3         0.4         1.7         2.9           26.7         2.7         21.3         31.0           4.4         1.3         2.5         7.0           2.5         0.8         1.3         4.4           10,394         781         9,333         11,698           99.9         6.8         88.9         112.9           0.60         0.04         0.52         0.68           0.31         0.02         0.28         0.35           0.83         0.05         0.74         0.92           0.53         0.05         0.45         0.61           17.9         1.6         14.9         21.1           11.9         1.1         9.6         14.1	Stand Mean         Std. Dev.         Min         Max         Among stands           2.3         0.4         1.7         2.9         58%           26.7         2.7         21.3         31.0         18%           4.4         1.3         2.5         7.0         55%           2.5         0.8         1.3         4.4         38%           10,394         781         9,333         11,698         18%           99.9         6.8         88.9         112.9         20%           0.60         0.04         0.52         0.68         32%           0.31         0.02         0.28         0.35         11%           0.83         0.05         0.74         0.92         46%           0.53         0.05         0.45         0.61         29%           17.9         1.6         14.9         21.1         10%

\*Variance componenent analysis based on 3 trees per stand

stand association of needle retention and a wood property for the entire population of Douglas-fir in the SNC hazard zone. The regression coefficients (b's) from random coefficient modeling at the within-stand level can be compared with the regression coefficients from the simple linear regression at the among-stand scale.

Our results indicate that SNC affects wood properties differently depending on the scale of analysis (tree-level or stand-level). At the amongstand level, reduced foliage appears to increase MOE and MOR, mostly through the increase in ring density, which in turn is a result of higher latewood proportion (Table 2, Figure 1a-b). No significant correlations existed between needle retention and density of individual ring components or MFA. At the tree-within-stand scale, needle retention (SNC) appears to impact only latewood MFA (Table 2); trees with more foliage had lower latewood MFA. However, this improved latewood MFA did not result in increased MOE or MOR.

Of particular interest is the relationship between ring width and needle retention. Trees infected with SNC produce narrower rings so the strong correlation between needle retention and ring width and rings/cm (Table 2, Figure 1c) is unsurprising. At the stand level, we also found a strong association between ring width and several ring properties, including ring density and latewood proportion (Table 3, Figure 2a). As with needle retention, we also ran the random coefficient model to better understand the relationship between rings/cm and wood properties within a stand. Because all trees had estimates of rings/ cm, we could produce a model with the full set of 179 trees. In both models, the increase in ring density with decreasing rings/cm was a function of the increasing latewood proportion. Although at the stand level no relationship was found between any MFA component and rings/cm, at the tree level earlywood MFA increased with ring width, resulting in increased overall MFA with increasing ring width. There was also a weak association between rings/cm and latewood density, with more rings linked to higher density.

Table 2. Results of correlation analysis and linear regression at the stand level and random coefficient modeling at the tree level for needle retention with wood properties.

Property	Sta	nd level (n :	Tree lev	Tree level (n=40)	
	r	b	Prob > f	b	Prob > f
Rings/cm (1x1 cm beam)	-0.67	-2.54	0.006	-0.53	0.46
Ring width (outer 2 cm of disk)	0.50	1.17	0.06	0.77	0.12
MOE	-0.42	-949.6	0.11	-258.4	0.68
MOR	-0.48	-9.15	0.07	-4.14	0.47
Density	-0.53	-0.06	0.04	-0.02	0.53
EW density	0.20	9.21	0.48	-7.24	0.71
LW density	-0.04	-5.46	0.89	56.09	0.12
MFA	-0.05	-0.15	0.87	-1.70	0.19
EW MFA	0.06	0.28	0.83	-0.99	0.57
LW MFA	-0.47	-1.45	0.08	-2.69	0.003
LW prop.	-0.66	-0.09	0.008	-0.05	0.22



Figure 1. Relationships between needle retention and (a) MOE and MOR, (b) ring density and latewood proportion, and (c) rings/cm. Each point is the mean for a stand.

Because increased density and lower MFA contribute to higher wood strength and stiffness, we also found increasing MOE and MOR with increased rings/cm at both the stand and tree level (Table 3, Figure 2b).

MOE and MOR are influenced by both density and MFA; consequently, any of the five sub-components that we studied-earlywood density, latewood density, latewood proportion, earlywood MFA, and latewood MFA-have the potential to affect MOE and MOR. We used multiple linear regression to model how these variables affect strength and stiffness using all of the individual tree data. Latewood MFA had no significant impact on MOE or MOR in the full models that included all five anatomical variables and was dropped from both models; all other components were significant at p  $\leq 0.01$ . Our data suggest that of these five anatomical properties, latewood proportion had by far the most significant influence on both MOE and MOR (largest tvalue) and earlywood density had the least influence. The impact of latewood proportion is not surprising because the difference between latewood density and MFA and earlywood density and MFA is far greater than any of the variation within earlywood or latewood (Table 1). The resulting equations are shown below:

MOE = 1525.73 + (4.99\*EW density) - (148.79\*EW MFA) + (7.26\*LW density) + (7440.18\*LW proportion) $r^{2} = 0.55$ 

$$\begin{split} MOR &= 0.48 + (0.05^* \text{EW density}) - (0.69^* \text{EW MFA}) \\ &+ (0.07^* \text{LW density}) + (77.64^* \text{LW proportion}) \\ r^2 &= 0.54 \end{split}$$

The path diagrams in Figures 3 and 4 demonstrate further how the sub-components of density and MFA contribute to the correlations between rings/cm and retention with MOE and MOR at the stand level.

In the diagrams, the association for each component is the product of the correlation between that component and rings/cm or retention, and the correlation between that component and MOE or MOR. Latewood proportion is clearly the driving factor behind the association of needle retention with MOE and MOR (Figure 3). The latewood proportion path products were –0.36 for MOE and –0.45 for MOR, accounting for the majority of the total correlations between needle retention and MOE (r = -0.43) and MOR

Table 3. Results of correlation analysis and linear regression at the stand level and random coefficient modeling at the tree level for rings/cm with wood properties.

	Stand level			Tree level		
		(n = 15	(n = 179)			
Property	r	b	Prob > f	b	Prob > f	
Ring width (outer 1 cm of disk)	-0.86	-0.53	0.001	-0.58	0.001	
MOE	0.77	447.79	0.001	433.68	0.001	
MOR	0.79	3.99	0.001	3.69	0.001	
Density	0.86	0.03	0.001	0.02	0.001	
EW density	0.02	0.16	0.959	1.21	0.621	
LW density	0.29	10.98	0.294	6.18	0.097	
MFA	-0.25	-0.21	0.370	-0.43 <sup>1</sup>	0.001	
EW MFA	-0.40	-0.46	0.141	-0.68 <sup>1</sup>	0.001	
LW MFA	0.44	0.37	0.103	0.06	0.694	
LW prop.	0.81	0.03	0.001	0.03	0.001	

<sup>1</sup>Did not converge with Proc MIXED; used Proc GLM.



Figure 2. Relationships between rings/cm and (a) MOE and MOR and (b) ring density and latewood proportion. Each point is the mean for a stand.

(r = -0.48). This demonstrates that stands with higher needle retention values had lower MOE and MOR, and that a decrease in latewood proportion was the primary driver of these relationships.

At the stand level, rings/cm had

stronger correlations with MOE and MOR (r = +0.767 and +0.789, respectively, Table 3 and Figure 4) than did needle retention. The path diagrams for rings/cm in Figure 4 show that latewood proportion is again the main factor behind this relationship, where trees with narrower rings had more latewood and thus stronger and stiffer wood. Other associations worth noting are rings/ cm with latewood density and with earlywood MFA: it appears that narrower rings are associated with higher latewood density and lower earlywood MFA, both of which cause MOE and MOR to increase.

#### Conclusions and Future Research

Trees in stands impacted by Swiss needle cast have higher MOE and MOR compared to trees in stands less affected by SNC, and this appears to be mainly a function of increased latewood proportion. These effects are correlated well with a decrease in ring width as a result of the disease. Within a stand, however, there is no association between needle retention of individual trees and MOE or MOR.

The next phase of our research (currently in progress, and funded by a grant from the USDA-FS Agenda 2020 Program) involves determining whether the trends we observed here are also present in healthy stands. Because of the close association between needle retention and ring width, it is difficult to separate the effects of the disease from possible growth rate effects. Results reported in the literature on growth



Figure 3. Path analysis for the stand-level relationships between needle retention and (a) MOE and (b) MOR.

rate effects on Douglas-fir wood properties are inconsistent. By looking at the relationships between needle retention, ring width, and wood properties in healthy stands, and combining the results from the results of this study, we will be able to determine whether Swiss needle cast affects ring properties differently than slow growth in general.

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Figure 4. Path analysis for the stand-level relationships between rings/cm and (a) MOE and (b) MOR.

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# Fungicidal Control of Swiss Needle Cast in a 20-Year-Old Forest Stand

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#### Abstract

Annual spray appplications of chorothalonil fungicide were carried out over five consecutive years from 1996 - 2000 in a Douglas-fir forest stand. Fungicide applications reduced infection levels of Phaeocryptopus gaeumannii compared to the unsprayed plots in foliage sampled in 2001. Total needle retention was also increased in the fungicide spray treatment in 2001. Effects of fungicide spray on P. gaeumannii levels persisted in foliage produced in 2001, which was not fungicide treated but had lower infection levels in the treated plots due to reduced inoculum levels compared to the unsprayed plots. Incidence of infection in the 2000 and 2001 foliage sampled in spring of 2002 was near 100% in the fungicide treated plots. Differences in total infection between fungicide treated plots and untreated controls were slight, suggesting that residual effects of fungicide treatment were short-lived. Foliage from the same plots was again sampled in spring of 2003. Overall infection levels averaged over three foliage age classes (2000–2002) remained significantly lower in the fungicide treated plots. Infection levels in 2001 foliage sampled in 2003 (two-year-old needles) were again significantly less than in the unsprayed plots. Infection levels were not significantly different for 2002 foliage sampled in 2003 (one-year-old needles), suggesting that the residual effect of fungicide treatment on inoculum reduction lasted only 2 years.

#### Introduction

Although the pathogen *Phaeocryptopus gaeumannii* is widely distributed on Douglas-fir in western Oregon and Washington, extensive and severe Swiss needle cast disease (SNC) has not been the historical norm. Severe SNC symptoms have been observed in the western Coast Range from about 1990. Management options for controlling SNC in forest plantations are very limited at present, and the potential for control of SNC by fungicides has not received much attention in the United States. The disease is controlled in Christmas tree plantations by the fungicide chlorothalonil (Chastagner and Byther 1982, Skilling 1981, Hadfield and Douglas 1982). Annual applications of chlorothalonil are recommended beginning three years prior to planned harvest for marketable Christmas trees (Chastagner and Byther 1982). However, because of the longer duration of protection needed for Douglas-fir rotations, aerial fungicide sprays have not been considered an economically effective option for control of SNC in forest plantations.
Whether SNC can be controlled in forest plantations by aerial applications of fungicides is not known. Some aspects of P. gaeumannii biology suggest that annual spray applications throughout a rotation might not be necessary for disease control. Needles are susceptible to infection only during their first growing season. Needles more than one year old are not susceptible to new infection by ascospores (Stone et al unpublished). Therefore, needles only need to be protected for a few weeks during the peak period of ascospore release, late May through mid June of the first growing season, to keep them disease free for their normal lifetime of four to five years. Effective spray applications for two or three consecutive years could eliminate or significantly reduce levels of infection and inoculum production. New, unprotected foliage produced in subsequent years could become infected from residual infections or from outside sources of inoculum.

If fungicide sprays are successful in reducing infections below a critical level, and thereby reducing the inoculum load, SNC might be controlled for several years beyond cessation of fungicide application in treated stands without additional fungicide applications. The amount of time before infection levels return to high levels would depend on the effectiveness of the fungicide spray, residual infection levels, proximity and severity of inoculum sources to the treated stands, and local environmental conditions favoring infection. Furthermore, most Douglas-fir trees can tolerate a low to moderate level of infection without developing symptoms of SNC. While it is probably impossible to completely eliminate *P. gaeumannii* from a forest stand by fungicide sprays, reducing and maintaining infection to below harmful levels may be possible.

This study was undertaken to determine whether a program of aerial fungicide spraying is effective in reducing infection levels of P. gaeumannii in forest stands, and how long the residual effect of fungicide treatment is maintained after the cessation of five consecutive annual fungicide treatments. This study was done in cooperation with the Oregon Department of Forestry, which treated study sites with annual aerial applications of Bravo fungicide between 1996–2000. Foliage produced in spring 2001 was the first year of untreated foliage in the fungicide treated plots. Foliage was again collected in the spring of 2003, and the 2001 and 2002 needle complements were sampled.

## **Materials and Methods**

#### **Study sites**

Aerial sprays were applied to a st udy site established by the Oregon Department of Forestry near Beaver, OR. The stand was established in 1980 and was characterized as severely diseased in 1995. Bravo Weatherstik 720 fungicide was applied at the rate of 5.5 pt/30 gal/ acre by aerial spray to three five-acre plots for five consecutive years, 1996-2000. Sprays were applied shortly after bud break, with shoots averaging 1–3 in. Adjacent unsprayed fiveacre plots were designated controls.

#### Sampling

Two transects were established in each of three plots that received fungicide treatment and three unsprayed control study plots. Transects were oriented perpendicular to each other and crossed the plots from edge to edge from approximately the mid point of each side. Sampling points were located at approximately 100 ft intervals along each transect starting at approximately 50 ft in from the plot edge. Each transect had 6 sample points except in plot T3, a long narrow plot, which had 8 points in one transect and 4 in the other. Two trees were sampled at each sample point, two secondary lateral branches were cut from the fifth to seventh whorl from the top. Needle retention on the sampled branches was assessed in the field, then branches were placed in bags and returned to the lab for infection assessments. Samples were collected from plots C1, C2, T1, and T2 in May 2001 and 2002, and from all six plots in May 2003.

#### Assessment

Needle retention was rated on a scale of 1–9, where 9 = 90-100% needles retained, for each internode starting with the current year (2001 foliage). Retention ratings for each internode (1997–2000) were summed to obtain a composite retention index for each branch. Needles were removed from internodes by age class, placed in envelopes and stored frozen (-20 C). Three samples of ten needles /age class/ branch/tree were randomly drawn

for quantitative PCR analysis (Winton et al. 2002). A sample of 50 needles/ age class/branch/tree was randomly drawn for pseudothecia counts. Needles were affixed abaxial side up to index cards with double sided adhesive tape. Cards were examined under dissecting microscopes to determine the proportion of needles bearing pseudothecia (incidence of infection). The first ten needles on each card with pseudothecia present were then used to determine pseudothecia density, or severity. The needles were examined under a dissecting microscope fitted with a counting grid and the proportion of stomata occupied by pseudothecia in three segments (base, middle, tip) of each of the ten needles was determined. Infection index, the product of incidence and pseudothecia density, was used as a response variable for comparisons of treatments. Since incidence of infection was near 100% for all plots, infection index mainly reflects differences in amount of pseudothecia on needles. Statistical analyses were performed with SAS for Windows Vers. 8 (SAS Institute, Cary, NC) and Statgraphics (Manuguistics Inc, Rockville, MD).

## Results

For foliage sampled in May, 2001, which had been treated for five consecutive years, needle retention was significantly greater in plots that had been treated with Bravo for five years (p < 0.05, Table 1). The average retention index for treated plots, 27.3, represents approximately 75% of foliage in age classes 1–4 retained compared to about 40% for the control. The distribution of

needles retained in the two treatments is shown in Figure 1. Nearly all current year needles were retained in both treatments. Nearly all 1999 needles were also retained in the Bravo treated plots, but only about half of this complement was retained in the unsprayed control plots. 70-80% of 1998 needles were retained in the treated plots compared to less than 10% for the control plots. A small proportion of 1997 needles remained on the treated trees but this complement was completely absent from the unsprayed trees.

Quantitative PCR (QPCR) analysis of foliage sampled in May, 2001 showed significant differences in levels of *P. gaeumannii* in foliage for age classes 1998-2000 (Table 2). There

 

 Table 1. Mean needle retention indices for treated and control plots. Indices are the sum of needle retention ratings for each of four internodes,

1997-2000 for foliage sampled	d in May 2001.
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Plot	<b>Composite Retention Index</b>
TI	28.7
T3	25.8
C1	15.9
C3	12.4

were too few 1997 needles present in the control plot trees for this age class to be included in the analysis. QPCR values for both fungicide sprayed plots were significantly lower than foliage from unsprayed controls for all three age classes sampled.

Differences between fungicide sprayed and control treatments persisted through the first year that aerial fungicide sprays were not applied to the treatment plots. Foliage that emerged in June 2000 was the last age class to be treated with fungicide spray. Age class 2000 foliage from fungicide treated plots had one-half to one-third the level of infection (infection index) present in the unsprayed control at two years following emergence (Table 3). Infection levels in age class 2001 foliage were also significantly greater in the unsprayed controls than in the fungicide treated plots, although differences were not as great. Infection levels in 2001 foliage in plot C1 (unsprayed) were not statistically different from the two sprayed plots (Table 3).

Incidence of infection, the proportion of needles bearing at least



Figure 1. Comparison of needle retention ratings for plots treated with Bravo fungicide for 5 years (T1, T3) and unsprayed (C1, C3). Ratings are based on field assessment of needle retention for each internode on secondary lateral branches taken from between the 5-7<sup>th</sup> whorl from the treetop in May 2001.

Table 2. Comparison of quantitative PCR values (ng *P. gaeumannii* DNA/pg Douglas-fir DNA) for foliage for fungicide sprayed and unsprayed plots measured in May, 2001. Letters in the same column indicate different groups at p < 0.05.

		Needle Age Cla	ss
Treatment	2000	1999	1998
C1	2.40 b	2.18 b	2.17 b
C3	2.56 b	2.38 b	2.22 b
TI	0.79 a	1.05 a	1.18 a
T3	0.28 a	0.46 a	1.14 a

Table 3. Comparison of infection index (incidence xpseudothecia density) for foliage collected in May2002. Letters in the same column indicate differentgroups at p <0.05.</td>

Plot	2001 Needles	2000 Needles
C1	0.12 b	0.30 b
C3	0.18 a	0.46 a
TI	0.08 b	0.14 c
T3	0.10 b	0.15 c

one pseudothecium, measured in May, 2002 was near 100% for fungicide sprayed as well as unsprayed plots for both age classes, indicating virtually all needles in both treatments and both age classes were infected. Incidence of infection was also near 100% for all needle age classes and treatments in foliage collected in 2003. Differeneces in infection index (NFX) therefore reflect differences in the abundance of pseudothecia (Table 3, 4). Nearly all one-year-old needles (2001 needles) in the 2002 sample were infected regardless of treatment, but the amount of infection in fungicide sprayed plots was slightly less than for unsprayed plots (Table 3). Pseudothecia levels in one-year-old needles (2002) collected in spring of 2003 from the fungicide treated plots, however, were not statistically different from the unsprayed plots (Table 4). Figures 2 and 3 show the trend of infection levels in fungicide treated plots gradually approaching levels in the untreated checks. Infection levels in two-yearold foliage from the fungicide treated plots were significantly lower than in the unsprayed plots in 2002, reflecting fungicide treatment of the foliage produced in spring of 2000.

Infection levels were also lower in two-year-old needles in 2003, reflecting the lower infection seen in this age class (2001) after the first year (Table 3), but the difference between sprayed and unsprayed plots was less than for 2002 (Figure 2). Infection levels in one-year-old foliage from fungicide treated plots were lower than from the unsprayed plots in 2002, but treatments were not different in 2003 (Figure 3).

### Discussion

Fungicide applications for five

consecutive years resulted in concurrent reduction of *P. gaeumannii* infection and significantly greater foliage retention in the sprayed com-

Table 4. Comparison of infection index (incidence x pseudothecia density) for foliage collected in May 2003. Letters in the same column indicate different groups at p < 0.05.

Plot	2002 Needles	2001 Needles	2000 Needles
(1	0.20 ABC	0.44 B	0.48 B
C2	0.15 A	0.40 B	0.50 B
C3	0.25 C	0.55 A	0.25 AB
TI	0.14 A	0.33 B	0.33 A
T2	0.16 AB	0.35 B	0.35 A
T3	0.21 BC	0.41 B	0.30 A



Figure 2. Comparison of infection index in two-year-old needles sampled in 2002 and 2003. Age class 2000 needles, which were two-years-old when sampled in 2002, were the last needles to receive fungicide treatment. Age class 2001 needles sampled in 2003 were not treated with fungicide, lower infection levels indicate the residual effect of fungicide treatment on inoculum levels in the fungicide treated plots.



Figure 3. Comparison of infection index in one-year-old needles sampled in 2002 and 2003.

pared to the unsprayed plots. Needle retention in current year needles was not different between sprayed and unsprayed plots, as expected. However, increased retention of the twoand three-year old needles resulted in approximately one additional years' complement of foliage in the sprayed vs. unsprayed plots in 2001, reflecting the effects of fungicide treatment on disease control. A separate analysis of wood characteristics from these plots showed that untreated control trees also have lower wood moisture content, decreased ring width, and increased latewood proportion compared to fungicide treated trees, characteristics that have been attributed to reduced vigor and defoliation due to disease (Johnson et al 2001). Taken together, these results reinforce the conclusion that P. gaeumannii is the primary agent responsible for the current decline of Douglas-fir in western Oregon and Washington. A separate analysis of growth from this study is in progress.

Foliage from fungicide treated plots had significantly lower levels of P. gaeumannii than unsprayed controls in 2001 foliage measured in May, 2002. Since this foliage had not been sprayed with a protectant fungicide, lower infection levels in this age complement must reflect reduced inoculum levels resulting from multiple year treatment in the fungicide-sprayed plots. However, it appears that differences in infection levels between sprayed and unsprayed plots after multi year treatment were already beginning to converge in the first complement of foliage not protected by fungicide. Infection levels in both treatment plots were significantly different from only one of the unsprayed control plots.

Infection levels in the fungicide treated plots continued to converge

toward levels in the untreated checks in 2003. Infection levels in the oneyear-old 2002 foliage from the two treatments were not statistically different. This suggests that even after five consecutive years of fungicide applications, sufficient residual infection remained in the stands to enable infection levels to rapidly build up in only two years after the cessation of fungicide treatment.

Even though chlorothalonil fungicide significantly reduced P. gaeumannii levels in this study and others, complete elimination of the pathogen from the stands was not expected. Detectable residual levels of infection typically remain even where good disease control has been achieved (Chastagner and Byther 1983, Chastagner and Stone 2001). The amount of residual infection following a course of treatment is probably the most important factor affecting long term disease control. The amount of residual infection will be determined by the efficacy of the treatment, coverage, and timing. In this study, the amount of residual infection appears to be relatively high. Chlorothalonil has proved effective in controlling SNC in Christmas trees (Chastagner and Byther 1983) and forest plantations (Chastagner and Stone 2001). The WeatherStik formulation of chlorothalonil (Daconil) provided superior control to other formulations (Chastagner and Stone 2001).

Although significant differences in infection levels were detected between treatments, our data suggest that the aerial fungicide spray treatment was only minimally effective in controlling *P. gaeumannii*. Incidence of infection in fungicide treated age class 2000 needles, measured in May, 2002 was nearly 100%, not significantly different from the unsprayed control. Even though the fungicide treatment reduced the amount of infection in these needles, there was still sufficient successful infection to effectively saturate foliage. Pseudothecia density in the sprayed plots was one-third to onehalf that of the untreated control and accounted for the observed differences between treatments.

The pronounced increase in needle retention in the fungicide sprayed trees is somewhat surprising given their relatively high levels of residual infection. Manter et al. (2002) estimate that at pseudothecia densities above 25% the net annual needle carbon budget is negative due to impaired photosynthesis. Mean pseudothecia densities for fungicide treated, age class 2000 foliage in this study were below this threshold necessary to trigger absicission, but not unsprayed 2000 foliage, in agreement with the prediction of Manter et al. Given that incidence of infection in fungicide sprayed foliage was near 100%, it is not surprising that the unsprayed 2001 foliage in the fungicide treated plots also was nearly 100% infected. Furthermore, although there was a significant difference between control and fungicide treatments in the 2001 foliage, only one of the treatment plots was significantly different from controls when analyzed individually. It therefore appears that the control provided by five years of protectant fungicide application may be breaking down only one year after cessation of fungicide applications.

Aerial application of a copper fungicide was ineffective in reducing P. gaeumannii infections in a 19vear-old forest stand of Douglas-fir in New Zealand. However, handspraying the same material at the same concentration reduced incidence of infection to below 42% compared to 100% in unsprayed control (Hood and van der Pas 1979). The success of the handspray application was attributed to superior foliage coverage and greater amounts of material applied. Handspray applications were applied to run off by a person climbing within the tree crowns. This highlights the difficulty of achieving sufficient aerial application rates in forest tree stands compared to Christmas trees, where SNC is routinely controlled by fungicide. The relatively high amount of residual infection present in the fungicide treated plots in our study suggest that coverage may have been inadequate. Fungicide application volumes for forestry should probably be adjusted based on stand age and foliage area rather than a standard rate per acre. It is also possible that the level of disease at this site was too severe, and that aerial fungicides might be more effective in controlling SNC at sites with more moderate levels of disease.

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## *Control of Swiss Needle Cast in Forest Plantations by Aerially Applied Elemental Sulfur Fungicide*

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## Abstract

A field study was established to evaluate the efficacy of aerially applied sulfur fungicide for control of Swiss needle cast in Douglas-fir forest plantations. Paired plots of five acres each were established at six sites in the Coast Range. Half the sites were 15-20 year old plantations that had been precommercially thinned, half were 20-25 years old and had been commercially thinned. One plot of each pair received sulfur fungicide treatment at the rate of 60 lb/acre, applied twice in a two-week interval in early June 2002. Aerial application of elemental sulfur fungicide resulted in increases in foliar percent sulfur content and SO<sub>4</sub> sulfur as well as increased P, Ca, Mg, Mn, Z, and B. Percent Fe was lower in sulfure treated foliage, percent N, K, C, and Cu were not different between sulfur treated and untreated foliage. Infection of 2002 foliage was reduced significanatly in all plot pairs. During 2002, an additional trial was established in Washington to determine the effectiveness of a single application of several other types of fungicides in protecting needles from SNC. Materials in this trial included Thiolux, several copper fungicides, a biologically-based material, and Daconil WeatherStik. Kocide and Daconil were the only materials that reduced SNC infection levels.

## Introduction

There are few management options for controlling Swiss needle cast (SNC) in forest plantations at present. Control of SNC in forest plantations has met with mixed success where it has been attempted. Aerial application of a copper fungicide was ineffective in reducing *P. gaeumannii* infections in a 19-year-old forest stand of Douglas-fir in New Zealand. However, handspraying the same material at the same concentration reduced incidence of infection to below 42% compared to 100% in unsprayed control (Hood and van der Pas 1979). The disease is controlled in Christmas tree plantations by the fungicide chlorothalonil (Chastagner and Byther 1982, Skilling 1981, Hadfield and Douglas 1982). Annual applications of chlorothalonil are recommended beginning three years prior to planned harvest for marketable Christmas trees (Chastagner and Byther 1982). However, because of the longer duration of protection needed for Douglas-fir rotations, aerial fungicide sprays

have not been considered an economically effective option for control of SNC in forest plantations. Furthermore, toxicity of chlorothalonil to fish and other aquatic organisms and its moderately long persistence in soil make it an unsuitable material for forest use.

Previous studies (Chastagner 2002, Chastagner and Stone 2001, Crane et al 2001, Stone et al 2000) have indicated that elemental sulfur formulations (Thiolux, Golden Dew) are moderately effective protectant fungicides for control of SNC. Although not as effective as chlorothalonil in reducing infection by Phaeocryptopus gaeumannii in field studies (Chastagner and Stone 2001, Stone et al 2000), elemental sulfur is classified as an EPA toxicity category IV material, the least toxic category. Sulfur is considered a very low toxicity material that poses a very low risk to human and animal health. Sulfur also has a very low toxicity to birds, fish and aquatic invertebrates and so its use in forestry applications is not subject to the same level of environmental concern as for chlorothalonil. Addtionally, some field observations have also suggested that in addition to its fungicidal properties, elemental sulfur can act as a nutrient in sulfur-deficient soils, resulting in improved foliage color and increased growth.

Evaluations of elemental sulfur fungicides for control of SNC have, to date, been conducted as simulated aerial applications on small trees. Because of the interest of forest managers in the potential for use of elemental sulfur for control of SNC in operational stands, a study was established to evaluate aerially applied sulfur on 15 to 25-year old Douglas-fir stands in the Coast Range.

2002 Fungicide screening trial (DF 102) - In addition to the aerial Thiolux studies, a trial was also established in Washington to examine the effectiveness of several types of fungicides in protecting needles from SNC (Table 3). Materials included in this trial are Daconil WeatherStik, Thiolux, several copper fungicides (Kocide DF, Kop-R-spray, and Phyton 27) and a biologically-based material (QRD 131). A single application of each material was applied to one tree in each of 10 blocks on May 31, 2002. All treatments were applied with a Solo backpack sprayer equipped with an 8003 nozzle @ 15 psi. Trees were sprayed to wet and the length of new growth ranged from 1.5 to 3.5 inches at the time of application. A non-sprayed tree in each block served as checks.

The effects of these treatments on disease development were assessed during spring 2003 by harvesting a single branch from the middle portion of each tree on March 10, 2003. Infection levels were assessed as described for the Washington aerial Thiolux study. In addition, needle loss and color were also rated. Needle loss was rated on a scale of 0 to 10, where 0 = none, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%,..., and 10 = 91-100% loss. Needle color was rated on a 1 to 6 scale, where 1 =healthy-appearing dark green needles, 2 = healthy-appearing green needles, 3 = needles with a slight yellow mottling on a green background that may also have brown

spots or tips on the needles, 4 = dull green needles with moderate chlorosis that may also have brown spots or tips on the needles, 5 = extensive yellowing/browning, and 6 = uniformly yellow needles that may have some brown spots or tips.

Table 3. Products included in f	Table 3. Products included in fungicide trials.					
Trade name and formulation	Active ingredient					
Daconil WeatherStik 720	chlorothalonil					
Golden Dew Sulfur 92%	sulfur					
Kocide DF	copper hydroxide, copper metallic equivalent 40%					
Kop-R-Spray	copper ammonium carbonate, copper metallic equivalent 8%					
Phyton 27	copper sulphate pentahydrate, copper metallic equivalent 5.5%					
QRD 131 AS	Bacillus spp.					
Tactic sticker	synthetic latex and organosilicone					
Thiolux 80W	sulfur					

#### Study sites

Four groups of study sites were established in spring of 2002 with the cooperation of Simpson Timber Company, Starker Forests, Rayonier Inc., and the Oregon Department of Forestry. Each group consisted of a plot pair established in a 10 - 15-year old plantation and a plot pair in a 20-25year-old plantation, except the Rayonier site which had two plot pairs in a 10-15-year old plantation only one plot pair. Each plot pair consisted of two 5-acre plots in the same stand (15 acre minimum) with a uniform topography. One plot of each pair received the aerial sulfur application, the other was an untreated check. Additional criteria for the stands were that they should be primarily stocked with Douglas-fir, the 10–15-year-old stands to have been precommercially thinned with a stocking of 300- 350 trees per acre, the 20–25-year-old stand to have been thinned within 3 years and have a stocking density of 200-250 tpa. One-half acre permanent study plots were established in the center of each five acre plot and all trees numbered and measured. Growth measurements will be made again in 2004.

## Sulfur fungicide application

Thiolux micronized elemental sulfur was applied to each treated plot by helicopter in early June 2002 at the rate of 60 lb/ac, as two passes of 30 gal/ac applied in two perpendicular directions. At 10 to 14 days after the first application, a second application was made at the same rate. Each plot received a total of 120 lb of Thiolux/ac.

## **Foliage collection**

In Oregon, foliage was collected manually by climbing ten randomly selected trees from each plot. Two branches from the fifth whorl from the treetop were collected, and 3-4tertiary lateral shoots were clipped and placed in collecting bags. At the Washington site, the tertiary lateral shoot samples were obtained from a single branch that was collected from 15 randomly selected trees in each plot using an extension ladder and pole trimmer. A collection of foliage was taken in fall 2002 after the first sulfur application for comparison of foliage elemental analysis. A second collection was taken in June 2003 for assessment of *P. gaeumannii* infection.

#### Analyses

Foliage collected in fall 2002 was separated into age classes, placed in paper bags labeled by site/ treatment/tree/foliage age and air dried. Elemental analysis was performed by the Central analytical Laboratory of Oregon State University. For assessment of infection by P. gaeumannii, at the Oregon sites needles were separated by age class, the needles for each age class pooled for each tree, and a sample of 50 needles randomly drawn for each tree/age class. The 50 needles were affixed to 3 x 5" index cards with double sided adhesive tape and examined under the dissecting microscope at 40x for presence of pseudothecia of P. gaeumannii. Incidence was scored as the proportion of needles bearing P. gaeumannii pseudothecia. Severity, the proportion of stomata occupied by P. gaeumannii pseudothecia, was determined from the first 10 needles bearing pseudothecia. The needles were examined under a dissecting microscope fitted with a counting grid and the proportion of stomata occupied by pseudothecia in three segments (petiole, middle, tip) of each of the ten needles was determined. Infection index, the product of incidence and severity, was used as a response variable for comparisons of treatments. Statistical analyses were carried out with the Statgraphics statistical package (Manuguistics Inc, Rockville, MD).

At the Washington site, infection assessments were done by visually examining needles on the different age classes of shoots using a dissecting microscope. The incidence of infected needles was rated on a scale of 0 to 10, where 0 = nopseudothecia evident on any needles, 1 = 1-10% of the needles with pseudothecia,... 10 = 91-100% of the needles with one or more pseudothecia. The severity of infection was rated on a 0 to 6 scale, where 0 = no pseudothecia present,1 < 1%, 2 = 1-10%, 3 = 11-25%, 4 = 26-50%, 5 = 51-75%, and 6 = >75% of the stomata occupied by pseudothecia. Again, an infection index, based on the product of the incidence and severity ratings, was used to statistically compare treatments using SAS Proprietary Software Release 8.1 (SAS Institute Inc, Cary, NC).

#### Results

#### **Nutrient analysis**

Foliage collected at both the Oregon and Washington sites five to six months following aerial sulfur application had higher percentage sulfur and higher amounts of sulfatesulfur than the unsprayed foliage, as would be expected. The sulfate-sulfur content of treated needles was almost two and a half times that of the unsprayed needles. Percent phosphorus, calcium, magnesium, manganese, zinc and boron were also higher in the sulfur treated foliage. Percent nitrogen, potassium, copper and carbon were not different between the sprayed and unsprayed foliage. Percent iron was less in the sprayed than unsprayed foliage (Table 1).

Table 1. Effect of sulfur application on foliage nutrient levels. 2002 foliage sprayed in June, 2002, collected and analyzed Nov, Dec 2002. Summary of all study sites (Oregon: ODF, Starker, Simpson. Washington: WSU/Rayonier). Two-factor ANOVA.

		Sulfur Trec	ıtment		
		Spray		No Spray	
Response	mean	SD	mean	SD	р
S percent	0.044	0.015	0.026	0.008	0.0000
SO <sub>4</sub> -S ppm	240.07	160.51	100.94	86.20	0.0000
N percent	1.71	0.248	1.65	0.196	0.0483
P percent	0.169	0.053	0.146	0.041	0.0000
K percent	0.780	0.159	0.772	0.169	0.7605
Ca percent	0.278	0.082	0.219	0.051	0.0000
Mg percent	0.126	0.041	0.110	0.030	0.0005
Fe ppm	50.50	17.15	53.70	11.60	0.0309
Mn ppm	117.71	26.68	106.63	33.04	0.0024
Zn ppm	13.21	4.69	11.27	4.24	0.0005
B ppm	21.53	7.38	18.95	6.02	0.0058
Cu ppm	4.76	1.06	4.45	0.93	0.0627
C percent	53.05	1.39	52.99	1.39	0.7516

#### Phaeocryptopus gauemannii infection

Aerially applied sulfur fungicide significantly reduced infection in one-year-old needles by Ρ. gaeumannii over all plots (Table 2, Figure 1). Differences in infection levels were significantly lower for the sulfur treated foliage for all plot pairs (Table 2, Figure 2). The magnitude of the difference in overall infection index varied from about 0.05 for the ODF No Womans Land site to more than 0.1 for the two Starker Forests plot pairs (Table 2). Incidence of infection, the proportion of needles bearing at least one pseudothecium, was reduced for all plot pairs except the Simpson Timber Co. 10-year-old plot pair (Figure 3).

Disease ratings for one- and twoyear-old foliage were analyzed for the Washington site in 2003. As expected, there was no difference between sprayed and unsprayed foliage for the 2001 foliage sampled in



Figure 1. Comparison of infection index (incidence x severity) in one-year-old Douglas-fir foliage from unsprayed and sprayed foliage treated with aerial applications of Thiolux fungicide in June 2002. Foliage was collected and analyzed in June, 2003. Bars are standard error.

spring 2003 (Table 4). For the 2002 foliage, however, infection by *P*. *gaeumannii* was significantly reduced (Table 4). Incidence of infection in the 2002 foliage was reduced (0.7 vs. 8.8).

2002 Fungicide screening trial (DF 102) -Only two of the treatments (Kocide @ 2lb and Daconil WeatherStik) significantly reduced the overall level of disease compared to the unsprayed check (Table 5). These were also the only two treatments that significantly reduced the incidence of needles with pseudothecia (Data not shown). Only a limited amount of needle loss was evident on the samples and there were no differences between treatments. Although most treatments had no effect on needle color, needles on trees sprayed with Kop-R-Spray had significantly poorer color than the needles on the unsprayed checks (Table 5).

Data from this trial confirms the effectiveness of a single ground based application of Daconil in controlling SNC, particularly under relatively low disease pressure. Of the other materials tested, Kocide appears to be an additional fungicide that may have the potential to control SNC. However, additional studies are needed to obtain a better understanding of

Table 2. Comparison of *P. gaeumannii* infection index (incidence x severity) in sulfur sprayed vs. unsprayed one-year-old foliage at Oregon sites. Significance levels are for a two-sample t-test comaprison of NFX means (sulfur vs. check) for 2002 foliage.

Site	Sulfur	Check	Difference	р
Starker 10-yr-old	0.0028	0.0696	0.0668	0.001
Starker 20-yr-old	0.0008	0.1216	0.1208	0.001
Simpson 10-yr-old	0.0734	0.1766	0.1032	0.009
Simpson 25-yr-old	0.0285	0.1067	0.0781	0.023
ODF No Womans 18 yr old	0.0007	0.0479	0.0471	0.001
ODF Kansas Creek	0.0107	0.1128	0.1021	0.001

optimal rates and the effect of multiple applications of this material on **Dist** disease development.



*Figure 2. Comparison of differences in infection index (NFX, incidence x severity) by site for one-year-old Douglas-fir foliage. Bars are standard error.* 



*Figure 3.* Comparison of differences in incidence of infection by site for one-year-old Douglas-fir needles. Bars are standard error.

Table 4. Comparison of disease ratings in sulfur sprayed vs unsprayed one-year-old and two-year-old foliage collected in June 2003 from the Washington site. Age class 2001 needles were one year old when the sulfur sprays were applied in June 2002.

Disease Index	Sprayed	Check	P value (t-test)
2001 needles	34.8	36.4	0.886
2002 needles	0.7	13.1	0.001

Table 5. Effect of a single application of fungicide in 2002 on the development of Swiss needle cast in 2003.

Treatment <sup>1</sup>	Product per 100 gallons	Disease index <sup>2</sup>	Needle loss <sup>2</sup>	Needle color <sup>2</sup>
QRD 131 AS	3 gal	14.0 a	0.1 a	2.4 ab
Check	-	13.0 ab	0.1 a	1.7 bc
Phyton 27	25 oz	11.7 abc	0.1 a	2.6 ab
Kop-R-Spray	2 qts	9.5 abc	0.0 α	2.8 α
Thiolux 80W	60 lbs	9.2 abc	0.2 α	2.0 abc
Kocide DF	4 lbs	8.3 abc	0.0 α	2.3 abc
Thiolux 80W	90 lbs	7.2 bc	0.0 α	1.6 bc
Kocide DF	2 lbs	5.7 cd	0.0 a	2.0 abc
Daconil WeatherStik 720	5.5 pts	0.2 d	0.0 a	1.3 c

<sup>1</sup> Each treatment was applied to a single randomly selected tree in each of 10 blocks on May 31. 2002 and the foliage was sprayed to wet.

<sup>2</sup> Disease index and needle color/loss data are for samples collected on March 10, 2003. Numbers in columns followed by the same letter are not significantly different, P=0.05, DMRT.

#### Discussion

Elemental sulfur is one of the oldest known pesticides. Its use to control plant diseases dates from at least 1800, although its pesticide properties were known as early as 1000 bc (Tweedy 1969). Sulfur is currently registered by the U.S. Environmental Protection Agency for use as an insecticide, fungicide, and rodenticide on several hundred food and feed crop, ornamental, turf and residential uses. It is also used as a fertilizer or soil amendment. Sulfur is applied in dust, granular or liquid form, and is an active ingredient in numerous registered pesticide products (Thomson 1993). Although it has long been used for control of fungal diseases, its mode of action against fungal cells is not fully understood. It is generally thought that sulfur interferes with oxidative phosphorylation (respiration) by acting as an electron acceptor from cytochrome b, thus interrupting the mitochondrial electron transport chain (Tweedy (1969). It is generally used as a protectant or contact fungicide, i.e. it has no systemic or eradicant activity. Elemental sulfur was inhibitory to *P. gaeumannii* ascospore germination and germ hypha growth at relatively high concentrations (Stone et al 2000).

Results of this study suggest that aerially applied elemental sulfur can be effective in reducing foliage infection by P. gaeumannii in forest plantations, and may be a useful tool for SNC management. In addition to its effect in reducing foliage infection by P. gaeumannii, elemental sulfur also increased foliage content of several elements, suggesting that it is also acting as a nutritional supplement. As expected, foliar content of  $SO_4$  sulfur was higher in the treated plots, but higher levels of P, Ca, Mg, Mn, and B in the treated foliage suggest that uptake of these elements was increased by sulfur applications.

Whether of not elemental sulfur will be of operation use will require further investigation and longer term study. The prohibitive expense of fungicide application has generally precluded their use for control of foliage diseases in forest plantations. A cost-benefit model taking into account plantation age, growth differential, fungicide application costs etc would be helpful to managers considering whether or not to invest in chemical control of SNC. A further consideration is that residual effects of fungicide treatment appear to be relatively short term. Phaeocryptopus infection levels were significantly reduced, and needle retention increased after five consecutive years of chlorothalonil fungicide spray in a coastal Douglas-fir

plantation. However, infection levels rebounded quickly after cessation of spray treatments and infection levels between fungicide treated and untreated plots were not significantly different two years after the final fungicide applications (see Stone et al, this volume). This suggests that fungicidal control of SNC can only be accomplished by continued fungicide application throughout a rotation. Unless increases in growth following control of SNC persist over a longer period of time, the use of fungicides to control SNC will probably not be cost effective. Additional research is needed to determine the effects of sulfur fungicide sprays on growth and how long increases in growth rates persist following the cessation of applications. may have utility for a short term improvement of growth in stands affected by SNC that are near harvest age.

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## Predicting Geographic Variation in Swiss Needle Cast Severity in the Coast Range Based on Winter Temperature

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## Introduction

Following the first reports of Swiss needle cast disease in Europe and the eastern U.S., Boyce (1940) reported that the causal agent, Phaeocryptopus gaeumannii, was widespread throughout the Douglasfir region of western North America but did not cause disease. In considering the contrasting behavior of P. gaeumannii on its host in western North America, where its effect was negligible, versus Europe, where it was a serious defoliating pathogen, Boyce (1940) reasoned that seasonal patterns in local climate could differentially affect fungal growth and development, and this could explain the virulence of P. gaeumannii in Europe and the eastern U.S. In particular, Boyce (1940) pointed to the warm, humid summers with episodic rain typical of continental Europe as being more conducive to fungal growth, in contrast to the more arid summer climate of the Pacific Northwest. Hood (1982) also found evidence of climate effects on the distribution of P. gaeumannii in southern British Columbia. Although Hood (1982) did not comment on disease severity, he observed a correlation between precipitation, particularly spring rainfall, and abundance of P. gaeumannii.

Several aspects of the current SNC epidemic in western Oregon also suggest that differences in local climate may be important determinants of disease severity. Accumulating evidence supports Boyce's (1940) suggestion that climate factors that favor growth of the fungus may allow it to reach epidemic levels, even in the area where it is native. The pathogen is widely distributed and abundant throughout Douglas-fir stands in the Coast Range, so it can be assumed that restrictions to dissemination of spores in Coast Range forests are at most very slight. Yet severity of disease ranges from minor in some stands to extremely severe in others. Therefore, presence of the fungus alone is not sufficient to cause disease. Some interaction between host, pathogen and environment is required for disease to develop. Interior variety Douglas-fir seed sources are known to be more susceptible to the disease than the coastal variety. However, the widespread distribution of the disease, estimated at 387,000 acres in the aerial survey conducted in 2002 by the Oregon Department of Forestry, which encompasses multiple ownerships, stand ages, and management histories, appears to rule out inappropriate seed sources as a primary cause of the current epidemic.

The pattern of spatial distribution of SNC in coastal Oregon observed over the past several years suggests a relationship between disease severity and climate. Observations by the Oregon Department of Forestry aerial survey, conducted annually since 1996, consistently have shown most severe disease to be in stands nearest the coast, gradually lessening inland. More severe symptoms (chlorosis, defoliation) on south aspect slopes has also been reported by various observers. Furthermore, disease levels at individual sites are relatively consistent from year to year. We have intensively monitored disease severity at nine sites in the Coast Range since 1996. While disease severity at these sites fluctuates somewhat from year to year, the relative disease severity among the sites has remained fairly constant (Figures 1, 2), suggesting that site-related factors are important determinants of disease severity.

Rosso and Hansen. (2003) investigated the relationship between SNC severity in Coast Range plantations and several climate and topographic variables by means of multiple regression analyses. Rosso and Hansen. (2003) found the strongest relationship ( $r^2 = 0.57$ ) between site disease rating and a regression model that included mean maximum July temperature, mean July preciptiation, July fog occurrence, and aspect. While Rosso's model explained about 60% of the observed variation in stand SNC rating, it was not successful in predicting defoliation (r<sup>2</sup>= 0.29).

In previous studies we have re-



Figure 1. Mean two-year needle retention averaged over 5 years and mean pseudothecial density (proportion of stomata occluded by pseudothecia) for one- and two-year old needles averaged over four years for nine Swiss needle cast study sites in western Oregon.



Figure 2. Relationship between four-year average needle retention and four-year average pseudothecial density (proportion of stomata occluded by pseudothecia) for nine Swiss needle cast study sites in western Oregon.

ported differences in fungal colonization between upper and lower canopies and between stands with southern vs northern aspects (Manter et al. 2003). Because the most severe disease occurs in sites within the low elevation coastal fog zone (so called because of the frequency of summer fog) the amount of free water on foliage in the summer has also been considered a possible factor affecting disease severity (Hansen et al. 2001, Rosso and Hansen. 2003). Since several climatic variables covary with these positional effects (temperature, light, relative humidity, and plant water content), we implemented a study to investigate several of these factors individually. The objective of this study was to evaluate the relative importance of climate variables by season to better understand their influence on different phases of the infection cycle and colonization of foliage by *P*. gaeumannii, and to use this to develop a descriptive model for distribution and severity of Swiss needle cast disease in western Oregon. Whereas Rosso and Hansen (2003) investigated the relationship between climate variables and disease symptoms in stands (based on a 1 to 6 rating system), the approach taken here is to examine the relationship between climate variables and abundance of *P. gaeumannii*.

## Methods

#### **Seedling studies**

Douglas-fir seedlings (Burnt Woods open-pollinated seed source, Starker Forests, Inc., Corvallis, OR) were exposed to natural inoculum at a moderately diseased coastal Oregon plantation (Salal plantation, Table 1) for 4 weeks in June 2000. After inoculation seedlings were relocated to Corvallis and incubated under different levels of shade, irrigation, and mist treatments. Postinoculation environment treatments were applied in a split-randomized complete block design with overhead mist and shade treatments applied to the whole block, and irrigation treatments applied to half the seedlings in the block. Mist treatments consisted of either no mist or three-2 hr mist treatments daily, shade treatments were 100 or 50 % of ambient light provided by a shade cloth suspended above the seedlings, and irrigation treatments were 1.9 or 0.5 L of water per day. Incubation chambers were 0.9 x 1.8 x 1.2 m frames constructed from PVC pipe to support shade cloth and mist tubing. Each chamber held 6 trees, and the two irrigation treatments were applied to 3 trees in each chamber.

Fungal colonization was determined at monthly intervals by visual assessments of the amount of *P. gaeumannii* pseudothecia (infection index) on needles. Needles were haphazardly collected from two current year internodes and pooled for each seedling. From this sample, 50 needles for each seedling were randomly drawn, affixed to index cards with double sided adhesive tape, and examined under the dissecting microscope to determine the proportion of needles bearing

Table 1. Characteristics of field plots.								
	Site		Disease		Mile to	Age	Seed	
Site	Code	Group	Severity	Elevation	0cean/bay	(2001)	Source	Aspect
Acey Creek	AC	North	Healthy	670	8	15	1600ft & 1400ft	E
Coal Creek	CC	North	Moderate	220	5	15	1600ft & 1400ft	SE
North Fork	NF	North	Severe	160	4.75	15	Boundary 1800ft	SW
Upper Stone	UP	Central	Healthy	1700	14.5	19	Boundary 1800ft	Ν
Lower Stone	LO	Central	Mild	430	14.75	19	Boundary 1800ft	SW
Juno Hill	JH	Central	Severe	380	2.25	19	Boundary 1800ft	NE
Limestone	LM	South	Healthy	890	12.25	14	1000ft	Ν
Cedar North	CN	South	Mild	1500	7.5	14	1000ft	NW
Salal	SL	South	Moderate	370	4	14	1000ft	NW

pseudothecia (incidence of infection). The first ten needles on each card with pseudothecia present were then used to determine the proportion of stomata occluded by pseudothecia (severity). The needles were examined under a dissecting microscope fitted with a counting grid and the proportion of stomata occupied by pseudothecia in three, 2.6 x 0.26 mm segments (base, middle, tip) of each of the ten needles was determined and averaged. Infection index (NFX), the product of the percent of needles with visible pseudothecia (incidence, n = 50) times average severity (n = 10), was used as a response variable for comparisons of treatments.

#### **Field Studies**

April measurements of P. gaeumannii colonization were made over a four-year period (1999-2002) at nine Douglas-fir plantations first established in 1996 (Hansen et al. 2000). The nine plots are grouped in three clusters of three plots each in the vicinity of Tillamook, Oregon (Table 1). The plots in each cluster were placed in Douglas-fir plantations of the same age and where possible the same seed source. Plantations were selected to represent a range of elevations, distances from the ocean, and disease severity. One plot in each group had moderate to heavy symptoms of SNC and one site was classified as healthy, although P. gaeumannii was present at all sites.

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

Each study site was equipped with a leaf wetness / temperature datalogger (Spectrum Technologies, Plainfield, IL), and observations were recorded hourly. Measurements were limited to May-November in 1998 and 1999, and then continuously through February 2002. Incidence, severity and infection index was determined annually for one- and twoyear-old needles taken from two lateral branches collected from the mid crown (fifth whorl below the terminal shoot) for each tree sampled per site. Samples were collected in April just prior to bud break.

## Analysis

Statistical analyses of differences in *NFX* between treatments in the post-inoculation seedling study were carried out by PROC Mixed with Tukey's multiple comparison procedures (SAS Vers. 8). Regression model selections were carried out using Statgraphics (Manugistics Inc., Rockville, MD).

## **Descriptive model**

Temperature data were obtained from the climate prediction model (www.daymet.org). DAYMET Daymet was developed at the University of Montana, Numerical Terradynamic Simulation Group (NTSG). DAYMET uses a digital elevation model and daily observations of minimum and maximum temperatures and precipitation from ground-based meteorological stations to produce an 18 year daily average data set (1980 - 1997) of temperature, precipication, humidity and radiation as a continuous surface at a 1 km resolution.

## Results

#### **Seedling studies**

Based on the March NFX measurements, ten months following exposure to inoculum P. gaeumannii colonization was reduced by both shading and misting (Fig. 3). Since all trees in the study received the same exposure to inoculum, the different post-inoculation conditions differentially affected the rates of foliage colonization and/or development of pseudothecia by P. gaeumannii (e.g., NFX ranged from 0.051 to 0.207. Despite a significant effect on plant water-stress (i.e., predawn xylem water potentials of -0.4  $\pm 0.1$  and  $-1.8 \pm 0.2$  MPa for the high and low irrigation treatments in August 2000, respectively), irrigation did not have a significant effect on levels of P. gaeumannii colonization (Fig. 3).

Figure 4 shows the effect of treatments on increase in pseudothecia



Fig. 3. Final Phaeocryptopus gaeumannii pseudothecia density values for seedlings exposed to shade, mist, and irrigation treatments. Sun = 100 % ambient, shade = 50% ambient, mist = three-2 hr overhead mist treatments per day, no mist = 0 overhead mist treatments per day, low irr. = 0.5 L day<sup>-1</sup> drip-irrigation, and high irr. = 1.9 L day<sup>-1</sup> drip-irrigation. Bars are the arithmetic means and individual standard errors. Treatment effects were analyzed using a split-randomized complete block design.

numbers over time for the shade and misting treatments. For all treatments, pseudothecia appearance followed an exponential growth curve, with the greatest increases occurring during the winter months of January and February. Differences in amounts of pseudothecia appearing in different treatments were first detectable in the November sample and continued to diverge during the winter and spring months. After ten months, pseudothecia numbers were highest in the no shade, no mist treatment, lowest in the shade with mist treatment, with intermediate levels in the other two treamtents. Since the pattern of pseudothecia abundance corresponded to treatment temperature rather than water availability, these results suggested that winter temperature might be the more important factor influencing the rate of foliage colonization by *P*. *gaeumannii*. Regression of final *NFX* against average daily winter temperature for the four treatments (December – February, Fig. 5) revealed a linear relationship between temperature and the amount of fungal colonization.



Fig. 4. Phaeocryptopus gaeumannii colonization levels over time for seedlings exposed to shade, mist, and irrigation treatments. Points are the arithmetic means and individual standard errors.



Fig. 5. Final Phaeocryptopus gaeumannii infection index (NFX) values for seedlings exposed to the four sun and mist treatment combinations in relation to mean-daily winter temperatures for the months of December, January, and February.

Table 2. Four-year summary of the percent of stomata occluded by *Phaeocryptopus gaeumannii* pseudothecia (infection index, NFX) in one- and two-year-old needles for nine coastal study sites

	One-year-old needles					Two-year-o	old needles	
Site Code <sup>2</sup>	<b>1999</b> <sup>1</sup>	2000	2001	2002	1999	2000	2001	2002
AC	0.026	0.074	0.028	0.016	0.115	0.196	0.165	0.154
((	0.055	0.097	0.063	0.024	0.227	0.252	0.227	0.157
NF	0.131	0.168	0.070	0.098	0.341	0.486	0.389	0.357
UP	0.040	0.030	0.017	-	0.181	0.209	0.149	-
LO	0.047	0.095	0.015	-	0.299	0.283	0.196	-
JH	0.212	0.247	0.166	0.163	0.548	0.525	0.470	0.393
LM	0.027	0.043	0.010	0.020	0.134	0.167	0.192	0.133
CN	0.015	0.023	0.010	0.070	0.101	0.178	0.203	0.125
SL	0.025	0.049	0.042	0.025	0.189	0.230	0.244	0.232

<sup>1</sup> Needles were sampled in April of the year denoted. Each value is the individual plot mean.
<sup>2</sup> See Table 1 for site abbreviation codes.

#### **Field Studies**

Disease levels over the course of the four-year sample period at the nine coastal Douglas-fir plantations are shown in Table 2. Amounts of Phaeocryptopus gaeumannii remained relatively constant within sites (e.g., ).163-0.24 and 0.07-0.23 for the sites with the highest and lowest NFX levels in one-year-old needles) and the relative ranking of plots by disease severity was consistent from year to year. Plot-specific climate data (total leaf wetness or LW, Table 3; average temperature or T, Table 4) were separated into three periods: spring, corresponding to the period when spore deposition and infection occur (May-July); summer, corresponding to the period of internal hyphal growth (August-October); and winter, corresponding to the period when development of pseudothecia occurs (December-February). The hierarchy of plots ranked by these factors also was consistent from year to year, although

with considerable annual variation, and paralleled the ranking by NFX. The annual averages for all plots in spring T were 12.52, 10.96, 12.43, and 11.59; summer T were 12.77, 12.25, 12.47, and 12.33; and winter T were N/A, N/A, 4.69, and 5.58 (Table 4). Between 1998- 2001, average spring LW for all plots ranged from 1276 in 1998 to 788 in 2001; for summer LW from 1301 in 1998 to 956 in 2001; and winter LW from 1260 in 2000 to 1475 in 2001 (Table 3). For the four years average spring Tranged from 10.96-12.52. Summer T was more consistent (12.25-12.77, Table 4).

Correlations between seasonal climate factors and *P. gaeumannii* infection index for the nine coastal sites are shown in Table 5. However, since differences in site-specific *P. gaeumannii* levels may influence disease levels, an indicator of site-specific annual inoculum potential was needed. Therefore, the following two correlations were performed: *NFX* in one-year-old needles was correlated

		Spr	ring <sup>1</sup>		Summer				Winter			
SiteCode	<sup>2</sup> 1998	1999	2000	2001	1998	1999	2000	2001	1998- 1999	1999- 2000	2000- 2001	2001- 2002
AC	1201	1079	930	563	1147	1059	1140	853	-	-	1207	1555
CC	967	1079	1082	669	1179	868	1030	749	-	-	1173	1449
NF	1456	1254	1240	1059	1593	1422	1393	1212	-	-	1586	1698
UP	1785	1302	1020	996	1237	999	955	795	-	-	-	981
LO	989	1228	1278	495	1193	1143	1212	678	-	-	1255	1517
JH	1212	1142	1234	693	1401	1176	1348	1057	-	-	973	1328
LM	747	1218	1109	819	1090	1148	1502	888	-	-	1195	1583
CN	1529	1446	985	665	1321	1136	1025	992	-	-	1417	1621
SL	1597	869	1294	1132	1545	1218	1343	1384	-	-	1277	1547

<sup>1</sup>Spring is the total number of hours for the months of May, June, and July; summer is the total number of hours for the months of August, September, and October; and winter is the total number of hours for the months of December, January, and February.

<sup>2</sup>See Table 1 for site abbreviation codes.

Table 4. Four-year summary of mean-daily temperatures (°C) for nine coastal study sites.

	Spring <sup>1</sup> Summer					Winter						
SiteCode	<sup>2</sup> 1998	1999	2000	2001	1998	1999	2000	2001	1998- 1999	1999- 2000	2000- 2001	2001- 2002
AC	12.73	10.87	13.57	12.17	13.17	13.50	13.50	12.33	-	-	4.17	4.87
0	13.00	11.57	12.80	12.80	13.43	12.87	12.80	13.00	-	-	5.10	6.00
NF	13.43	11.77	13.73	12.30	13.07	12.07	13.60	12.57	-	-	5.10	6.63
UP	11.17	9.93	11.93	10.53	12.23	11.93	11.83	11.13	-	-	-	-
LO	13.00	11.40	12.60	9.77	13.17	12.47	12.70	14.07	-	-	4.47	5.23
JH	12.57	11.40	12.43	12.27	13.07	12.43	12.80	12.70	-	-	6.30	8.90
LM	12.45	10.47	11.73	11.77	12.20	11.83	11.57	11.80	-	-	3.77	4.07
CN	11.73	10.20	11.17	11.00	12.23	11.60	11.47	11.60	-	-	3.87	3.77
SL	12.60	11.07	11.90	11.70	12.40	11.57	11.97	11.80	-	-	4.73	5.20

<sup>1</sup>Spring is the average for the months of May, June and July; summer is the average for the months of August, September, and October, and winter is the average for the months of December, January, and February.

with *NFX* in two-year-old needles, both of which were assumed to be indicators of inoculum potential for each site (e.g., 1998 needle cohort sampled in April 1999 correlated with 1997 needle cohort sampled in April 1999). Correlations with climate data were performed using the appropriate annual climate measurements for each needle age class measured (e.g., *NFX* for one-yearold 1998 needle cohort was correlated with 1998 climate data, and *NFX* for the two-year-old 1998 needle cohort was correlated with 1999 climate data).

For both the one-year-old and two-year-old needle infection indices, only the previous year infection index and winter *T* consistently showed high correlations (Table 5). When the relationships between infection indices for one- and two-year-old needles and winter *T* were plotted for each year, the slopes of the regressions varied by year (Fig. 6), suggesting that some other factor was also influ-

encing infection. Therefore, we added the relevant disease components (i.e. *NFX* in one- and two-year old needles in the previous year) to the predictive models for *NFX* in one-year-old and two-year-old needles. As a result, the predictive abilities were improved and allowed for a general equation that could be used to predict both one- and two-year-old *NFX* levels independent of year (Fig. 7).

We then used the *NFX* prediction models to retrospectively simulate the predicted historical pattern of *P. gaeumannii* levels in coastal Oregon plantations. The first simulation shows the theoretical pattern of *P. gaeumannii* colonization over time with a constant winter temperature (5.13 °C) and a starting infection index of 0.01 (Fig. 8). In this scenario, we see a rapid increase in *NFX* levels, which reach a stable maximum within five years. The second simulation is based on historical

Table 5. Pearson correlation coefficients (r) for all data.	Year is the needle
age class with appropriate weather data <sup>1</sup>	

			Pear	son's r	
Variable 1	Variable 2	1998	1999	2000	2001
PD (one-yr)	Spore load index	0.950	0.952	0.906	0.941
	T – winter	-	-	0.959	0.933
	T – summer	0.429	0.297	0.426	0.521
	T – spring	0.330	0.718	0.291	0.378
	LW – winter	-	-	-0.456	-0.464
	<i>LW</i> – summer	0.451	0.360	0.289	0.289
	LW — spring	-0.025	-0.135	0.402	0.116
PD (two-yr)	PD (one-yr)	-	0.956	0.919	0.849
	T – winter	-	-	0.873	0.888
	T – summer	-	0.083	0.418	0.465
	T – spring	-	0.683	0.298	0.361
	LW – winter	-	-		
	LW – summer	-	0.537		
	LW – spring	-	0.683		

<sup>1</sup>PD is pseudothecia density; LW is leaf wetness hours; T is mean daily temperature; winter is December, January, and February; spring is May, June, and July; summer is August, September, and October. Spore load index is the PD of the previous age class.
<sup>2</sup>See Table 1 for site abbreviation codes.



Fig 6. Regression between Phaeocryptopus gaeumannii infection index values for (Panel A) one- and (Panel B) two-year-old needles and average winter temperatures for nine field sites used for monitoring SNC levels in the Coast Range.



Fig. 7. Observed vs. predicted Phaeocryptopus gaeumannii infection index using the best two predictors for (Panel A) one-year-old [NFX=-0.104 + 0.0031 x X<sub>1</sub> +  $0.0157 x T_w$ ] and (Panel B) two-year-old needles [NFX= 0.0486 + 0.0114 x X<sub>2</sub> +  $0.0203 x T_w$ ] for nine field sites used for monitoring SNC levels in the Coast Range. X<sub>1</sub> is the index of spore load, X<sub>2</sub> is NFX for the modeled age class one year earlier, and T<sub>w</sub> is average mean daily winter temperature for the months Dec-Feb.



weather data from Tillamook, OR (Tillamook 1W, 358494, National Weather Service). Once again *P. gaeumannii* levels initially increase rapidly to equilibrium, then fluctuate with annual differences in winter temperature (Fig. 9). Note the predicted increase in *NFX* in one and two-year-old needles beginning around 1994. Figure 10 shows the general relationship between aver-

Fig. 8. Simulation of Phaeocryptopus gaeumannii infection index over time using the best-fit model for one-year-old (closed symbols) and two-year-old (open symbols) needles. Mean-daily winter temperature was held constant at 5.13 JC and the index of inoculum load was initially set to 0.01. The simulation equations are described in Figure 7. age winter temperatures and the predicted *NFX* levels. The vertical lines represent the observed maximum and minimum values for the nine SNC study plots in the Oregon Coast Range.



Fig. 9. Simulation of Phaeocryptopus gaeumannii infection index over time using the best-fit model for one-year-old (closed symbols) and two-year-old (open symbols) needles and historical climate data from the Tillamook 1-W weather station (station # 358494). The simulation equations are described in Fig. 7.



Fig. 10. Simulated final Phaeocryptopus gaeumannii pseudothecia densities using the best-fit model for one-year-old (closed symbols) and two-year-old (open symbols) needles over a range of constant mean-daily winter temperatures. Vertical lines represent the high (8.90 °C) and low (3.77 °C) mean-daily winter temperatures observed from the nine coastal study sites. The simulation equations are described in Figure 7.

#### **Disease prediction maps**

The relationships between NFX in one- and twoyear-old needles and average daily winter temperature shown in Fig. 10 were then used to generate disease prediction maps based on the DAYMET 18-year averages at a 1 km resolution for a portion of the Oregon Coast Range. The prediction maps allow graphical representation of the spatial distribution of P. gaeumannii in one- and two-year-old foliage (Fig. 11), and corresponding needle retention (Figure 12). The predicted distributions for one- and two-year-old needle retention were corrected for aspect following the equations in Manter et al. (2003), i.e. for north aspect NR=98.932-109.2 NFX; for south aspect NR=98.932-161.6NFX.



Figure 11. Estimates of needle retention for SNC-infected Douglas-fir in northwestern Oregon. Needle retention was calculated indepedently for north-and south-aspect slopes from predicted infection indexes.



Figure 12. Climate-based estimates of Phaeocryptopus gaeumannii colonization in northwestern Oregon. Infection indexes (NFX) were predicted from average daily temperatures (Dec-Feb). Temperature data is the historical average (1980-1997) estimated by the DAYMET model.

## Discussion

A descriptive model based on winter average mean daily temperature and current infection levels in one- and two-year-old needles is proposed for predicting SNC disease severity in the Oregon Coast Range. The use of average winter mean daily temperature in the model is supported by experimental observations on timing of peak growth by P. gaeumannii. It is hypothesized that low winter temperatures are the primary environmental factor limiting growth of P. gaeumannii in Coastal Oregon forests. Other factors that have been identified in laboratory studies as important in the infection cycle, such as high humidity and free water on needles during the initial infection period, are assumed not to be limiting at critical periods in the infection cycle in Coast Range forests.

An initial hypothesis was that shade and mist, factors generally regarded as more conducive to fungal growth due to their effect on RH, would favor more rapid growth of P. gaeumannii. Although sun, mist, and irrigation treatments were the primary factors tested, our results suggest that temperature may be the principle factor influencing the rate of fungal colonization of foliage. Temperature differences between the treatments alone can account for the observed differences in Ρ. gaeumannii growth. This result is consistent with previously observed patterns of greater fungal colonization in positions where relative ambient temperatures are warmer (Manter et al. 2003). For example, these field studies showed that southaspect plots, which were exposed to greater solar radiation, only increased fungal colonization in coastal Oregon sites, not in a Willamette Valley site. This pattern is consistent with warmer observed mean temperatures, rather than insolation, being the more important factor. Thus, microclimatic differences and small temperature changes have already been observed to be correlated with *P. gaeumannii* fungal colonization rates.

A laboratory study has shown that P. gaeumannii hyphal growth requires free moisture and has a maximum growth rate at temperatures near 18 °C (Capitano, 1999). In the current study, however, misting treatments actually reduced P. gaeumannii colonization levels. The apparent discrepancy between field and laboratory-culture studies may be best explained by moisture being non-limiting in the field studies. The current treatments were applied approximately one month after initial infection took place (i.e., mid-June), when the supply of ambient moisture on the needle surface was adequate to support germination and infection. Germ hyphae penetrate through the needle stomata and begin to grow internally - where internal needle moisture contents (i.e., 100 % RH) are sufficient for fungal growth. The importance of free moisture is probably limited to periods when hyphal growth is active on the needle surface, such as (i) during the infection process and (ii) the second cycle of growth or when pseudothecia initials produce surface hyphae (Capitano 1999).

Leaf wetness alone, however, is not a good predictor of *P. gaeumannii* 

colonization in the field. At our nine coastal SNC study sites, measurement of leaf wetness at a temperature range where P. gaeumannii should be physiologically active (12-26 C) revealed differences between sites, and the cumulative leaf wetness at 12-26 C from April-August was second best climatic predictor of needle colonization by P. gaeumannii. Spring-summer leaf wetness at 12-26 C therefore appears to be a good predictor of SNC severity and could be used as an additional criterion for evaluating suitability of sites for Douglas-fir growing where free moisture may be more limiting.

The winter temperature model was used to generate a disease prediction map to depict the spatial distribution of *P. gaeumannii* and SNC in Coastal forest plantations. The disease prediction map provides a basis for testing, validating, and improving the accuracy of the model. A partial map of potential SNC distribution in western Oregon is presented here for comment and testing. It should not be interpreted as showing actual disease levels, but rather of disease potential under current conditions. It should be stressed that the proposed model can only be applied to current conditions present in the Oregon Coast Range. We hypothesize that currently there is both a regional and local contribution to P. gaeumannii spore deposition that is not directly included in our chosen index of spore load. In areas where the regional contribution is low or not present, the current models will probably overestimate NFX levels. Thus, even in sites with similar winter temperatures the same level of P. gaeumannii colonization may not be realized and/or the time to reach the temperature-dependent *NFX* level may be increased.

An interesting property of the winter temperature model is that it predicts a rapid increase in infection levels to their equilibrium level for a disease-free site given low initial infection levels, as shown in Figure 8. A similar scenario has been observed in a series of test plots installed by the Oregon Department of Forestry (see Stone et al. p 34). In this study, a series of 5 ac plots were treated annually with an aerially applied fungicide (Bravo Weatherstik 720) for five consecutive years. Fungicide treatments reduced the P. gaeumannii infection levels to less than 90%, but once spraying was terminated, colonization levels were not different from the unsprayed plots after two years.

The winter temperature model helps explain differences in the spatial distribution of SNC in the Coast Range based on relative amounts of P. gaeumannii. The climate model does not alone explain the abrupt appearance of SNC in the Coast Range, which dates from about 1990. The regional disease problem is probably dependent upon at least the following two factors: (i) an increase in Douglas-fir basal area leading to a 'regional' spore load, and (ii) a warming trend over the last twenty years. In regard to the former, in 1997 the Oregon Department of Forestry (unpublished data) investigated the history of 76,970 ha of Douglas-fir plantations 10 - 30 years old growing within 29 km of the north Oregon Coast. About 31% of these plantations had been established on sites where hemlock and spruce had dominated the previous stand. Only 20% were on sites dominated by Douglas-fir in the previous rotation. The remaining areas were mostly alder stands that had been converted to Douglas-fir. Although historical records are scant, this at least suggests that Douglas-fir is more abundant in the coastal forests than earlier this century. As a result, fungal spores, which can travel long distances, may be dispersed from neighboring Douglas-fir stands leading to a regional contribution to inoculum load - one that may not exist elsewhere or did not exist in the past.

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

Subtle climatic differences that are primarily responsible for the different vegetation composition of the P. sitchensis and T. heterophylla zones also are likely important factors in disease severity. Hood (1982) found higher levels of P. gaeumannii in southern British Columbia and western Washington in coastal forests of Vancouver Island and the Olympic Peninsula, with lower levels in the rain shadow of eastern Vancouver Island and the interior. In our plots, disease symptoms are more severe and fungal colonization greatest in sites with low elevation near the coast. At slightly higher elevations and further inland, plantations of the same age and seed source have milder symptoms of disease and needle retention of 3 to 4 years on average. The fungus is still abundant, but predominantly on the older needles. Environmental differences between such nearby sites are subtle, but perhaps significant. Temperatures are milder and annual rainfall is actually lower closer to the coast in the Picea sitchensis Zone than it is at higher elevations in the Coast Range.

Further study is needed for validation, testing and refinement of the winter temperature model, especially for evaluating a broad range of sites in western Oregon. Our goal is to provide a tool that can be of use to land managers for selecting appropriate tree species for Coast Range plantations, and that can be used to predict the spatial distribution of SNC for assessing growth impacts.

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## Carbon Assimilation and Growth Modelling Studies

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## Objective

To develop a model for estimating annual net  $CO_2$  assimilation  $(A_{net})$  and tree volume growth that is suitable for a wide variety of disease levels and meterological conditions. Validate the robustness of estimated  $A_{net}$  to predict growth across a range of Coastal Douglas-fir plantations.

## Introduction

A more-detailed version of this model, developed using three Douglas-fir plantations with various levels of Swiss needle cast (SNC), showed that estimated annual rates of whole-tree net carbon assimilation were significantly correlated with observed tree volume growth. However, this version of the model requires intensive meteorological data (hourly time-step) that may not be readily available to clients interested in using it. Therefore a simpler version utilizing readily available climate data, *Phaeocryptopus gaeumannii* colonization (pseudothecia density), and tree size (diameter at breast height, DBH) was developed. The utility of such a model is that it provides forest managers with a tool to predict site-specific (i.e. climate driven) growth rates of Douglas-fir trees that account for the presence of SNC.

## Methods

## **Model Basics**

Annual net CO<sub>2</sub> assimilation (A<sub>net</sub>) was modelled for 1998 using a modified version of the gas exchange model previously developed for SNC affected trees (Manter et al., 2003). Briefly, this model estimates needle-level gas exchange (stomatal conductance and A<sub>net</sub>) based on meteorological data and the amount of stomata occluded with *Phaeocryptopus gaeumannii* pseudothecia with the assumption that stomata regulate water loss such that tree water potentials do not fall below a critical threshold (e.g. Bond and Kavanagh, 1999). In the current version, the model is run for an average day for each month and then summed up to a total of 365 days. The underlying meteorological calculations include daily patterns of: (i) solar radiation, based on site characteristics (latitude, longitude, aspect, and slope); (ii) air temperature, assumed to follow a sine-wave between the user supplied temperature minima and maxima with a period defined by solar day length; and



(iii) vapor pressure deficit, determined by assuming that daily temperature minima are equal to the dew point temperature (VPD = 0) (Jones, 1992; McMurtrie, 1993). Finally, needle-level estimates of gas exchange are scaled-up to the wholetree level based on previously derived relationships between leaf area, DBH and *P. gaeumannii* presence (Manter et al., 2003a, 2003b).

#### **Model Development and Validation**

A subset of 40 Douglas-fir plantations (ODF permanent growth plots) was used to develop a relationship between estimated annual Anet (kg tree<sup>-1</sup>) and observed volume growth (ft<sup>3</sup> tree<sup>-1</sup>) for the year 1998. Volume growth measurements at these plots have been previously reported (Maguire and Kanaskie, 2001). Anet was estimated using concurrent measurements of P. gaeumannii presence on one tree per plot, and publicly available temperature data. For example, at each plot, MTCLIM Vers 4.3 (http://www.ntsg.umt.edu) was to estimate monthly used temperature maxima and minima based on the closest available state weather station data (http://www.ocs.orst.edu). The remaining 28 ODF plantations were then used to compare predicted versus actual volume growth measurements for 1998.

## Results

Figure 1 shows the relationship between actual 1998 volume growth measurements and estimated  $A_{net}$ . As expected a highly significant relationship between carbon assimilation and growth was observed. Furthermore, a significant 1:1 relationship was observed for the subset of 28 plots used to validate the Anetderived estimates of volume growth (Figure 2). One of the benefits of this approach to document and model SNC's impacts on Douglasfir growth is that it accounts for both disease and climatic impacts on host physiology. For example, Table 1 shows a range of modelled growth estimates for ten theoretical plantations whose temperature regimes are defined by the historical temperature regimes (1962-2002) documented at the ten listed weather sta-

tions. As shown, even when disease levels are held constant, growth estimates may range from 1.0 to 2.08 ft<sup>3</sup> tree<sup>-1</sup>; therefore, modelling approaches that do not account for climate may not be accurate across a range of sites that have different climate regimes.

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Figure 1. Relationship between volume growth and estimated total net  $CO_2$  assimilation for 40 coastal Douglas-fir plantations for the year 1998.



Figure 2. Relationship between actual and estimated volume growth for 28 coastal Douglas-fir plantations for the year 1998.

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Table 1. Influence of climate on growth estimates of Douglas-fir with the same level of *P. gaeumannii* infection. Historical temperature data (1961-2002) from ten western Oregon weather stations were used to model annual  $A_{net}$  and growth; all other input variables were held constant. *Phaeocryptopus gaeumannii* colonization levels were set at 0, 10, and 25 % stomata with visible pseudothecia for the current, 1 and 2 yr old needles; latitude = 45°N; longitude = 124°W; slope = 45°, Aspect = 0°; and DBH = 20cm. Percent change is the change in growth compared to the Tillamook estimate.

Station Name	Actual Latitude (dd)	Actual Longitude (dd)	Actual Elevation (m)	Modelled growth (ft <sup>3</sup> tree <sup>-1</sup> )	Percent Change (%)
Laurel Mt	44.917	123.567	1094	1.00	-44.8
Newport	44.583	124.050	43	1.78	-1.7
Falls City	44.850	123.433	128	1.79	-1.1
Tillamook	45.450	123.867	3	1.81	0
Astoria	46.150	123.883	3	1.84	1.7
Otis	45.033	123.933	46	1.85	2.2
Corvallis	44.633	123.200	70	1.87	3.3
Forest Grove	45.533	123.100	55	1.89	4.4
Cloverdale	45.200	123.900	3	1.95	7.7
Elkton	43.600	123.583	37	2.00	10.5
Powers	42.883	124.067	70	2.08	14.9

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## Alterations in Douglas-fir Crown Structure and Morphology Due to Swiss Needle Cast in the Oregon Coast Range

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## Introduction

Plants respond to defoliation in many different and complex ways, depending on their growth habit and form, as well as the extent and duration of the defoliation. Swiss needle cast (SNC) inflicts its damage on Douglas-fir by disrupting physiological functions in needles and causing premature loss of foliage. Ensuing changes in total foliage amount and its age-class and vertical distribution cause growth losses and changes in crown and stem morphology. These changes are important from an economic viewpoint, but conversely may suggest tree or foliage dimensions that can serve as effective indices of SNC severity. The objectives of this study, which constituted the senior author's M.S. thesis, were: (1) to test whether SNC has a significant effect on general parameters of crown morphology, such as crown length, crown width, and crown profile (vertical pattern in branch length and diameter; (2) to test and quantify the effect of SNC on the number of primary and secondary branches produced by Douglas-fir; (3) to test and quantify the effect of SNC on needle size and total foliage amount, age-class distribution, and vertical distribution; and (4) to quantify the change in growth efficiency across a range in SNC severity.

## Methods

Data were collected from 22 of the permanent plots established as part of the SNCC Growth Impact Study (p 25), entailing destructive sampling of 82 trees adjacent to the plots. A set of trees at each plot was measured for dbh, height, and height to crown base, and then felled for branch sampling. Height and basal diameter of all live branches were recorded on the felled tree. Two whorl branches and one interwhorl branch were then randomly selected from each third of the live crown length, providing nine sample branches per tree. These branches were removed from the bole, bagged, transported to the lab, and kept cool until they were clipped, rebagged into annual age classes and dried. Total foliage and woody mass was determined for each branch, branch-level predictive equations were developed for each site, and the total amount of foliage by age class was estimated for each branch and the entire tree, as was total branchwood mass. A two-parameter ß-distribution was fitted to describe the vertical distribution of foliage both in total and by age



class. A series of linear and nonlinear regression equations were developed to test hypotheses regarding response of trees to defoliation.

### Results

SNC has led to crowns that are shorter than normal, although projected crown width remains unaffected. Crown radii and maximum branch diameter profiles suggested that changes within the crown might be occurring at different levels due to variations in SNC damage and response within the crown. The crowns become more conical relative to the paraboloid shape of healthy trees, and the relatively greater longitudinal branch growth at the base of diseased crowns causes the height of maximum crown width to be at or close to crown base, vs. 1/3 of the way up from crown base, on average, in healthy trees (Fig. 1).

The number of primary interwhorl branches decreased with greater disease severity, as did the number of secondary branches attached to the axis of primary branches. Both the lower density of foliage and the decline in branch frequency contributed a lower bulk density of crowns under severe SNC.

SNC has resulted in foliage that is smaller in length, width, and projected area and, hence, foliage that is lower in dry mass but higher in specific leaf area. Consistent with the symptoms of the disease, younger age classes of foliage comprise a larger portion of the total foliage mass of the trees, although the total mass is much reduced (Fig. 2). The current and 1-year-old needles are concentrated relatively higher in trees with severe SNC than in healthy trees, while the 2-, 3- and 4-year-old needles are concentrated further toward the base relative to healthy trees (Fig. 3).

Growth efficiency, defined as stem volume growth per unit of needle area, increased with increasing SNC severity. The rapid decline in net assimilation rate with increasing needle age is well documented, so this result is most likely caused by the dramatically younger average age of surviving foliaged on severely



Figure 1. Average crown profile for two trees with different SNC severity.



Figure 2. Relative and absolute distribution of foliage by age class for Douglas-fir trees with severe and light SNC intensity.



Figure 3. Vertical distribution of Douglas-fir foliage by age class for trees with severe and light SNC intensity.

impacted trees. However, total leaf mass and leaf area index of the stand is sharply reduced as SNC intensity increases, causing stand-level growth to decline.

## Conclusions

Defoliation causes significant changes at multiple levels. These changes occur at the individual needle, branch, tree, and stand levels, complicating our ability to accurately predict all growth and morphological responses of the trees to defoliation. The growth losses associated with SNC are due not only to the reduction of foliage area, but also to changes in the size, physiological condition, and vertical distribution of this foliage. Crown assessment indices such as foliage retention and crown length to sapwood area ratio represent different aspects of crown condition, and pseudothecia counts represent still another aspect. Integrating complementary measures of crown condition into forest models should therefore improve predictions of response by representing a greater array of processes by which SNC exerts its influence.

## Interactive Effects of Swiss Needle Cast and Commercial Thinning on Douglas-fir Growth and Development

Doug Mainwaring, OSU; Doug Maguire, OSU; Alan Kanaskie, ODF; and Jeff Brandt, ODF

## Introduction

Many Douglas-fir (*Pseudotsuga menziesii*) stands in western Oregon are suffering from Swiss needle cast (SNC), a foliage disease caused by the fungus *Phaecryptopus gaeumannii* (Hansen et al. 2000). Although this fungus is endemic throughout the range of coastal Douglas-fir (Boyce, 1940), there have been noted increases in fungal presence, infection incidence, disease symptoms, and associated negative growth effects in the last 10 years (Hansen et al., 2000). A recent aerial survey indicated that approximately 157,000 hectares out of the 1.2 million ha surveyed in Oregon showed detectable discoloration, an increase from the previous high of 119,000 hectares in 1999 (Kanaskie et al., 2002).

Of greatest concern are the approximately 76,000 hectares of 10-30 year old plantations in north coastal Oregon. These plantations exhibit various degrees of SNC infection, but severe SNC may prevent them from attaining merchantable size. An ongoing six-year study of this population has found that stands with the most severe levels of infection are experiencing a cubic volume growth loss of approximately 52%, with a population average of 21% volume growth loss (Maguire et al. 2002). Some heavily infected stands at the low end of this age range, believed to be growing so poorly that they have no chance of becoming merchantable, have been underplanted or cleared and replanted with non-susceptible species, in most cases western hemlock (*Tsuga heterophylla*).

Regeneration harvesting has usually been done in stands with tree size and value sufficient to cover costs of the operation. In these stands, thinning has been avoided as a tool to improve crown vigor because field observations and limited data suggest that thinning stands with severe Swiss needle cast may accelerate symptom development and associated growth declines. While evidence suggests that pre-commercial thinning of SNC-infected Douglas-fir even under severe SNC does not intensify disease development in younger stands (Kanaskie et al. 2002), the thinning response of older stands (30-60 yrs old) with varying degrees of Swiss needle cast damage is largely unknown.

Thinning is a key component of the Oregon Department of Forestry's (ODF) structure-based management plan (Oregon Dept. of Forestry 2001). Designed to meet changing public priorities, the new management guidelines call for 40-60% of each district's land base to be made

up of stands having a layered, or two-storied structure. (Oregon Dept. of Forestry 2001). Qualitative description of the structural components necessary to meet the criteria for these layered stands suggests that thinning will be an essential tool to produce the required structural diversity.

However, SNC may be a serious obstacle to meeting these objectives. Foresters currently must make complex stand management decisions about the feasibility of thinning under the possibility of subsequent SNC intensification, but have little data to support their decisions. Monitoring the response of stands to partial cutting across a range of initial Swiss needle cast intensity is essential to the overall ODF monitoring program and to the successful implementation of ODF's Northwest Forest Plan.

This study addresses the retrospective phase of a larger project whose objectives are to assess the effect of commercial thinning on disease symptoms and tree growth in stands with different initial levels of Swiss needle cast. The retrospective study targeted 30-60 yr-old stands thinned between 5 and 10 years ago, with the intention of testing for a connection between thinning response and the current level of SNC infection. Specific objectives were to test the following hypotheses: 1) volume and basal area growth both before and after thinning werelower where current SNC is severe; and 2) response to thinning declined with increasing current intensity of SNC. This retrospective phase cannot answer the question as to whether thinning itself exacerbates SNC or whether growth response to thinning

depends on initial SNC intensity. However, an ongoing permanent plot phase of the study will eventually be able to address these questions.

#### Methods

#### Study sites

The study sites were distributed across six different northwestern Oregon ODF districts (Tillamook, Forest Grove, Astoria, West Oregon, Coos, Clackamas-Marion), five of which manage land in the Coast Range. The Clackamas-Marion district, is located entirely within the Cascades, and was included due to evidence of a light infection level in this province (Freeman 2002).

Plots were distributed from 43.5°to 46.16° N latitude and from 124.06°-122.31° W longitude, and from 30 to 800m above sea level (Fig. 1). Over the last 40 years in this region, the mean January minimum was 32°F and the mean July maximum was 80°F. Total annual precipitation during this time averaged 60-120 inches, with approximately 70% of this falling from October to March.

#### **Field work**

Forty-five fixed area plots were established in northwestern Oregon during the winters of 2001 (24 plots) and 2002 (21 plots) (Table 1). These plots were measured during the dormant season to reconstruct annual basal area growth over a growth period twice as long as the time since thinning.

Plot locations were distributed across a range of disease severity classes and residual densities, and included different aspects and slopes. Target stands were 30 to 60 years of age, with at least 75% of the basal area in Douglas-fir, and had undergone commercial thinning 4 to 10 years ago. Plot locations were chosen from among candidate timber sales provided by the six ODF districts.

In a representative part of each stand, a square, 0.5 acre plot was established. On each plot, all trees >5 cm were tagged and measured for DBH (nearest 0.1 cm), and a subsample of 40 Douglas-fir were measured for total height, and height to lowest live branch (nearest 0.01 m). This subsample included the 10 largest Douglas-fir by dbh and the 4 smallest by dbh, with the remaining 26 distributed evenly across the diameter range of the plot. All Douglas-fir were cored for sapwood width



Figure 1. Plot locations

Table 1: Average attributes for plots in retrospective thinning study.

	0.05	Distance	<i>a.</i>	22.4	QMD-DF	Foliage	<i>c</i> i <i>c</i> i	Douglas-fir basal area	Basal area in			Years
Plot	ODF district	from coast (miles)	Site index (ft/50y)	RD* (at thin)	(at thin) (inches)	retention (yrs)	CL:SA (cm/cm <sup>2</sup> )	(at thin) (ft²/ac)	other species (ft²/ac)	Age (yrs)	Year measured	since thinning
Archer	Till	20	132.9	33.1	14.7	2.15	10.66	126.72	2.08	40.3	2003	5
Barsky	WO	25	151.9	34.8	15.4	3.9	6.92	136.56	2.7	40.9	2003	8
Bcamp	FG	28	99.4	28.3	14.7	3.48	7.23	108.55	0	49.3	2002	9
Beaver	FG	29	115.5	19.8	17.4	3.58	4.56	82.81	0	50.3	2003	10
BRidge	Coos	10	120.4	30.5	16.6	2.38	6.22	116.92	6.9	45.4	2002	5
BS20	Till	21	130.6	17.4	15.6	2.86	7.2	68.78	0	39.8	2002	6
BS35	Till	21	139.8	36.8	14.3	3.13	12.39	139.26	0	41.7	2002	6
Cope20	Till	15	132.9	19.1	15.4	3.52	5.72	62.25	10.81	34	2002	7
Cope35	Till	17	140.4	28.2	12.9	2.38	10.95	99.19	1.83	37.7	2002	6
CampM	Till	19	127.6	21.41	12.6	2.83	6.81	75.93	0	40.8	2003	10
Cbf	WO	19	143.7	31.1	13	3.13	10.78	112.12	0.6	35.1	2002	5
Cbs	WO	21	123.7	33.2	12.7	2.85	9.87	117.13	0	34.6	2002	5
Cedar	Till	16	136.2	29.1	13.5	2.1	9.54	103.67	2.88	37.6	2002	5
CedSum	Till	17	112.9	23.7	12	1.95	12.09	82.07	0	37.0	2003	6
Cochran	FG	26	137.1	41.6	19.8	3.6	5.97	174.68	6.48	54.5	2002	6
Cole	Ast	12	107.6	45.3	18.5	2.11	13.55	193.62	0.23	62.5	2002	4
Efork	CM	80	120.4	20.1	21.1	3.23	8.68	92.43	0.48	63.0	2003	5
Fox	Till	16	124.7	33.7	16.3	2.75	7.51	135.91	0	45.1	2002	6
Gbasin	СМ	82	105	33.4	12	3.1	10.97	114.43	0.24	40.1	2002	4
Guts	Till	14	119.8	15.9	20.3	1.96	7.2	71.79	0	47.9	2002	5
Hamlet	Ast	13	136.2	23.8	22.1	2.4	8.26	111.6	0.48	69.3	2003	5
Joe1	Coos	11	145.3	31.2	14.1	1.95	5.96	117.22	0	30.3	2003	5
Joe3	Coos	12	154.9	24.4	12.8	2.48	9.09	87.25	0.89	29.3	2003	5
KFH	Till	16	136.8	37.8	11.1	3.38	11.79	126.32	17.46	30.9	2003	6
KFL	Till	16	142.7	39.1	11.4	2.25	18.05	131.9	0	34.5	2003	6
KFalls	Till	14	131.9	25.9	13.3	2.34	8.99	94.35	0	35	2002	5
KiLo	Till	15	131.9	36.4	12.4	2	11.27	128.5	0	34.2	2002	7
Lyda	FG	25	134.8	35.3	15.3	3.43	9.87	138.04	0	42.7	2003	6
Mfalls	Till	14	113.8	34.2	20	1.63	8.32	152.76	0	52.7	2003	6
MoonE	Till	19	128.3	21.3	12.4	2.5	5.46	74.92	0	34.3	2003	7
MoonW	Till	17	133.2	33.7	11.3	3.13	10.01	113.08	5.77	32.1	2003	7
Moot	Till	8	115.8	28.7	18.1	2.19	11.12	110.69	0	54.5	2002	5
Mrph	Till	21	126.3	31.7	15.7	2.78	6.64	121.4	3.72	39.8	2002	7
PDX	Till	21	130.6	28.8	14.6	2.6	7.1	110.03	0	41.3	2003	9
Rock	FG	27	90.9	36.6	13.6	3.45	7.66	134.77	9.88	59.7	2003	5
Sbloom	Till	15	146	23.9	10.9	1.88	8.87	78.8	5.03	28.3	2003	5
Sleep	FG	28	140.7	34.9	18.6	4.38	6.37	146.62	1.55	52.5	2002	8
Smill	CM	71	104.3	48.4	14.7	2.55	11.97	183.65	0.84	56.3	2002	6
Stburn	FG	28	96.5	31.7	13.9	3.65	6.18	88.12	30.78	42.3	2002	8
Stmpot	Till	19	137.1	29.6	15.6	2.6	7.44	116.87	0	53.8	2002	5
TB	Coos	12	126.6	33.2	13.4	2	7.02	121.62	0	33.1	2002	4
TB03	Coos	11	134.8	32.3	12.5	1.65	8.86	114.08	1.98	33.4	2003	5
Wport	Ast	5	118.8	45.3	13.3	1.8	11.4	151.33	16.05	36.8	2002	4
Xmas	CM	78	146	28.8	14.3	3.93	7.38	108.77	0.78	33.1	2003	5
Ybay	Ast	5	118.4	39.9	13.5	1.65	12.66	146.32	5.71	35.5	2003	5
Μαχ		82	47 9	48 4	56 1	4 38	18.05	44 45	30 78	60 2		10
Min		5	97.7	150	97.6	1 695	4 56	14.70	0.0	טיי. 28 פ		4
Mean		22.9	38.9	31.0	37 7	2 70	8.9	26.65	3.03	42.3		6
					÷			20.00				-

\*RD = Curtis' (1982) relative density

and radial growth over a growth period twice as long as the time since thinning. Sapwood area at crown base was estimated using a previously constructed sapwood taper equation for Douglas-fir (Maguire and Batista 1996). In addition, ages were obtained for the 10 largest Douglasfir. The 10 largest trees were given foliage retention ratings as an estimate of SNC infection severity. The standard SNC rating is the number of years that foliage is retained, averaged across the upper, middle and lower third of the crown. Due to the height of crowns and associated visibility problems in these older, larger trees, a single rating was given for the whole tree, based on average retention of the crown.

#### Data analysis

Three different variables were computed to assess growth response to thinning: 1) Periodic annual volume and basal area increment; 2) annual percentage volume growth; and 3) post-thin to pre-thin basal area growth. Although basal area increment was measured directly, volume increment required estimating past height growth of the site trees from site index equations (Bruce 1981). Letting t be the time since thinning, the estimated heights in years 2001-t and 2001-2t were combined with the tree basal area backdated to 2001-t and 2001-2t to calculate an average volume to basal area ratio (VBAR) for each year (2001-2t, 2001-t, and 2001) and each plot. Plot volumes were calculated by multiplying each VBAR by plot basal area.

To isolate the "effect" of current

SNC severity on growth reponse to thinning, the response variables above were regressed on foliage retention and crown sparseness (CL/ SA = live crown length/crown base sapwood area; Maguire and Kanaskie 2002), in addition to other covariates that typically influence stand growth. The latter variables included Douglas-fir basal area, basal area of other species, site index, relative density, quadratic mean diameter, crown ratio, crown length, and indicator variables representing location and site effects.

## Results

#### Periodic annual volume increment

Approximately 88% of the variation in the logarithm of periodic annual volume increment was explained by the following model (MSE of 0.00978):

Taple 2	Parameter	estimates	for	model	[1]
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Variable	Parameter Estimate	Standard Error
b <sub>0</sub>	3.80125	0.86968
b	0.15364	0.06637
b <sub>2</sub>	-0.33803	0.10924
b <sub>3</sub>	0.01081	0.00193
b <sub>4</sub>	0.73945	0.06852
b <sub>5</sub>	-0.39435	0.21907
b <sub>7</sub>	-0.00571	0.00281

tention and CL:SA. Expected volume growth losses experienced by stands having lower levels of foliage retention and higher levels of crown sparseness than a "healthy" stand (Forest Grove district averages) range up to 36% (Fig. 2).

As with basal area growth, the parameter estimate for relative density (Table 2), indicated that individual trees have a greater response with heavier thins given similar lev-

[1]  $\ln(\text{VOLPAI}) = b_0 + b_1 \ln(\text{FOLRET}) + b_2 \ln(\text{CLSA}) + b_3 \text{SI} + b_4 \ln(\text{RD}) + b_5 \ln(\text{CL}) + b_6 \ln(\text{AGE})$ 

where VOLPAI = Periodic annual volume increment since thin  $(m^3/ha/yr)$ 

- FOLRET = Current average foliage retention for the plot (yrs)
- CL:SA = Current crown length to sapwood area ratio (cm/cm<sup>2</sup>) SI = 50 yr site index (ft)
- RD = Relative density immediately after thinning (Curtis 1982)
- CL = Current crown length (ft)
- AGE = Current breast height age (yrs)

Parameter estimates (Table 2) indicated that stand-level periodic annual volume increment increased with increasing foliage retention, site index, and relative density and decreased with increasing crown sparseness, crown length, and age.

Volume growth varies dramatically across the range in foliage reels of foliage retention. In short, the growth losses associated with SNC in these thinned stands, were similar to those previously reported for unthinned stands (Maguire et al. 2002).

#### Annual percentage volume growth

The model describing the loga-



Figure 3. Average annual percentage volume growth implied by model [3], assuming a CL:SA of 8.9 cm/cm<sup>2</sup>, a site index of 124.6 (ft, 50 yrs.), a crown length of 54.7 ft, a crown ratio of 0.535 (average of all 45 plots), and an RD of 35.



Figure 2. Volume growth loss implied by model [2], assuming a maximum foliage retention of 3.78 years, a maximum crown sparseness of 6.4 cm/cm<sup>2</sup>, and assuming a site index of 124.6 (ft, 50 yrs.), a crown length of 54.7 ft, (average of all 45 plots), and an RD of 35.

rithm of average annual percentage volume growth over the period since thinning explained approximately 93% of the variation in this response (MSE=0.00865):

101			
[2]	$\ln(AVOL) = b_0$	+ b	$b_1 \ln(FOLREI) + b_2 \ln(CLSA) + b_3(RD) + b_4(SI) + b_5 \ln(CL)$
	$+ b_6(CR) + b_7 li$	n(A	GE)
	where AVOL	=	% Annual volume growth (since thin)
		=	[(volume in 2001)/(volume in 2001- <i>t</i> )] <sup>1/t</sup> -1
	FOLRET	=	Average foliage retention for the plot (yrs)
	CL:SA	=	Crown length to sapwood area ratio (cm/cm <sup>2</sup> )
	RD	=	Relative density immediately after thinning (Curtis 1982)
	SI	=	50-yr site index (ft)
	CL	=	Crown length (ft)
	CR	=	Crown ratio (expressed as a decimal)
	AGE	=	Breast height age (years)

Parameter estimates (Table 3) indicated that average annual percentage volume growth increased with increasing foliage retention, site index, and crown ratio, and decreased with increasing crown sparseness, relative density, crown length, and age.

Stands with low foliage retention (1.63 years) have an implied annual average volume growth of no greater than 4% following thinning (Fig. 3), with a lower value probable, considering the negative correlation between foliage retention and CL:SA (Maguire and Kanaskie 2002). Similarly, expected volume growth overestimates for stands with low foliage retention would also suggest that % volume growth would be even lower. When compared to the nearly 5% growth rates exhibited by healthy stands (high foliage retention, low CL:SA stand) with average covariate values, the worst case 3% growth rates duplicate the nearly 40% volume growth loss indicated in Fig. 2.

Table 3.	Parameter	estimates	for	model [	2].
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Variable	Parameter Estimate	Standard Error
b <sub>0</sub>	3.24164	0.68558
b	0.19436	0.06396
b <sub>2</sub>	-0.32588	0.10877
b <sub>3</sub>	-0.00734	0.00236
b <sub>4</sub>	0.00610	0.00284
b <sub>5</sub>	-1.09815	0.36064
b <sub>6</sub>	1.27813	0.60596
b <sub>7</sub>	-0.73112	0.23709

Table 4.	Parameter	estimates	for	model [3].	
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Variable	Parameter Estimate	Standard Error
bo	1.31275	0.69418
b,	0.22002	0.09661
b <sub>2</sub>	-0.01406	0.00357
b <sub>3</sub>	0.00455	0.00172
b <sub>4</sub>	-0.38975	0.16966

# Ratio of post-thin/pre-thin basal area growth

Approximately 41% of the variation in the ratio of post-thin/pre-thin basal area growth was explained by the following model (MSE = 0.026): foliage retention levels below 2.1 years, post-thin basal area growth is less than pre-thin growth, and decreases at an increasing rate as foliage retention decreases. It is also evident that at low foliage retention levels, heavier thinning or lower re-

- [3] BagRat =  $b_0 + b_1 ln(FOLRET) + b_2 RD + b_3 SI + b_4 ln(CL)$ 
  - where BagRat = Ratio of BAG (PostBAG/PreBAG)
    - FOLRET = average foliage retention for the plot (yrs)
    - RD = Relative density immediately after thinning (Curtis 1982)
    - SI = 50-yr site index (ft)
    - CL = Crown length (ft)

Parameter estimates (Table 4) indicated that in stands thinned between four and ten years ago, the post-thin/pre-thin basal area growth ratio increased with increasing foliage retention and site index and decreasing relative density and crown length.

Represented graphically (Fig. 4), the model indicates that at an RD of 35, post-thin basal area growth equals or exceeds pre-thin growth (over equal time periods) at foliage retention levels of 2.1 years and above. At sidual stand density does stimulate a greater basal area growth response.

To gain perspective on expected ratio values, ratios were calculated for twenty stands for the period just prior to thinning. Twenty plots were thinned a sufficiently long time ago that two 3-yr growth periods were available prior to thinning. For trees in these plots, the basal area growth was computed for these two periods and the ratio was calculated by dividing the three-year basal area growth just prior to thinning by the



Figure 4. Post-thin/Pre-thin basal area growth ratio implied by model [4], applicable to sites other than those on the West Oregon district. Squares represent the basal area growth ratio for the two successive 3-yr growth periods prior to thinning.

three-year basal area growth prior to that period for all residual trees on a plot. The latest season of growth used for this comparison was 1997, and the average current foliage retention of the stands used in this comparison was 3.1 years, suggesting limited influence of SNC. The average basal area growth ratio for the twenty plots was 0.92, and ranged from 0.50 to 1.13 (Fig. 4). Given that the model-implied ratio at an RD of 35 and a foliage retention of 1.63 years is 0.95, the growth of the residuals in such a stand is little better than the same trees would be in a higher density stand about to be thinned.

#### Discussion

Comparison of growth rates in Fig. 3 with growth rates from other thinned plots provides some perspective on relative growth rates in stands unaffected by Swiss needle cast. The Levels-of-Growing-Stock installation near Hoskins, Oregon has helped define relationships between density and growth in repeatedly thinned stands. Percent cubic volume growth in the medium thin (six years after thinning at breast height age 29) was 6.2%, with the stand having an RD of 48. At the same age, the unthinnned control plot, with an RD of 95, was growing at an annual rate of 2.4%. When the stand attributes from the medium thinning at age 35 (Marshall and Curtis 2001) were entered into equation 3 to predict % volume growth, the estimated annual volume growth was 6.0% when a foliage retention of 3.5 years and a CL:SA of 5.0 cm/cm<sup>2</sup> was assumed. Had this same stand had a foliage retention of only 1.65 years and the study average CL:SA of 8.9 cm/cm<sup>2</sup>, the calculated growth percentage would be 4.2%. While such a heavy SNC presence would likely have an effect on the other crown covariates, these percentages seem realistic given the high site quality of the Hoskins site.

The use of the post-thin/pre-thin growth ratio makes it possible to evaluate the response of a stand to thinning. Because the ratio is based on equal periods of time prior to and after thinning, a value greater than 1 indicates that growth has accelerated in the residual trees over the specified time period and a value less than 1 indicates deceleration in growth. It is important to emphasize that this ratio only reflects the prethin growth of the residuals rather than growth of the entire pre-thinned stand.

Although this study only allows current SNC levels to be used to estimate growth responses to thinning, these results provide some insight into the utility of a conventional thin in stands where SNC infection is significant. Even at low foliage retentions, basal area growth of individual trees after a heavy thin may be significantly greater than prethin levels. Equation [4] implies that a stand with a foliage retention of 1.94 years and a relative density of 25 would have an individual tree basal area growth response equal ratio to a "healthy" stand (having a

foliage retention of 3.65 years) thinned to an RD of 35. For an equivalent response in the residual trees, a stand with the lower foliage retention of 1.63 years would need to have been thinned to an RD of 22.5. Although the response to thinning does decline with increasing SNC severity at the end of the post-thinning period, individual tree growth can apparently be accelerated by thinning, although this conclusion assumes that SNC severity is not influenced by thinning. The permanent plot phase of this project is needed to confirm this assumption or quantify any change in SNC evoked by thinning.

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# Growth of Young Forest Stands after **Balanced** Fertilization

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## Introduction

Forest fertilization experiments in Pacific Northwest forests have identified nitrogen as the most nutrient that most commonly limits tree growth. Many companies and some agencies have supported or still support operational fertilization, predominantly with nitrogen applied as urea. More recently, interest has been renewed in the concept of balance among nutrients and corresponding balanced fertilizers that blend macro- and micro-nutrients, often prescribed on the basis of foliar chemistry. A little over five years ago a group of individuals from several companies collaborated on a balanced fertilizer trial in young Douglasfir stands. Because Swiss needle cast was recognized as a problem in parts of the targeted region, the trials also offered the opportunity to test effects of the nutrient amendments on disease symptoms.

# Objective

The initial and primary objective of the collaborative effort was to test the response of young Douglas-fir stands to balanced fertilizer additions. A secondary objective was to test the response of foliage retention to fertilization with alternative formulations.

# Methods

Study plot locations were distributed across a range of soil productivities, elevations, and aspects in the central Oregon Coast Range and west slope of the Cascade mountains. Two age classes were investigated: unthinned Douglas-fir planted 8-12 years ago, and previously-thinned Douglas-fir plantations of 20-30 years of age (Table 1).

Each unit had three separate fertilizer treatments as well as an untreated control. The three fertilizer treatments included: 1) nitrogen only, 2) nitrogen, phosphorus, and potassium (NPK), or 3) nitrogen, phosphorus, potassium plus other nutrients (NPKplus). In addition, the NPK and NPKplus treatments were tailored to the severity of Swiss needle cast (SNC). Plots visibly affected by SNC were given a higher level of potassium than plots unaffected by SNC. Fertilizer ingredients and levels are shown in Table 2.



Tab	le 1		Some	attribut	es of	locations or	bloc	ks	receiving	32	or	40	ferti	lizatio	n p	lots	ŝ
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			Date		Site	Number of	SNC
Unit	Name	Owner	planted	TPA	Index	miniplots	impacted?
1	Underhill	Starker	1967	197	130	40	Y
2	Harlan	Van Eck	1972	627	140	40	Y
3	Lightning tree	Starker	1981	326	136	40	Y
4	Toledo	Van Eck	1991	488	125	40	Y
5	1000 Line	Van Eck	1977	342	125	40	Y
6	Bayview	Van Eck	1977	461	125	40	Y
7	Coal Creek	CTC	1976	209	110	32	N
8	Marks Ridge	CTC	1967	205	130	40	N
9	Cold Springs	Weyerhaeuser	1965	206	100	32	N
10	Dutch Creek	Weyerhaeuser	1985	320	120	32	N
11	Swede Hill	Simpson	1985	475	120	40	Ŷ
12	East Newton	Plum Creek	1981	404	132	40	Y
13	Fall Creek	Plum Creek	1984	384	130	40	Y
14	Rocky 20	Plum Creek	1989	369	113	40	Ŷ
15	Fairview	Plum Creek	1987	611	133	40	Y
16	Kutch Creek	Weyerhaeuser	1983	413	110	40	N

Table 2. Fertilizer treatments with active rate (lbs./acre) of elements. High K treatments were applied at locations where Swiss needle cast symptoms were obvious, low K treatments elsewhere.

	NPK low	NPK high	NPKplus, low	NPKplus, high	
Nutrient	K, (trt 1)	K, (trt 2)	K, (trt 3)	K, (trt 4)	N (trt 5)
N	200	200	200	200	200
Р	60	60	60	60	-
К	110	250	110	250	-
Mg	-	-	50	50	-
S	-	-	80	80	-
В	-	-	2	2	-
Cu	-	-	10	10	-
Fe	-	-	5	5	-
Zn	-	-	15	15	-
Ca	-	-	50	50	-

The different fertilizer treatments were randomly assigned to miniplots encompassing 0.025 acres in 8-12 year old stands, and encompassing 0.065 acres in 20-30 year old stands. Of the 16 locations or blocks, 13 contained 10 miniplots for each of the four treatments (40 plots total) and three locations contained 8 miniplots for each of the four treatments (32 plots total), giving a total of 616 miniplots or experimental units (Table 1). Double coverage of fertilizer mixes were applied with a rotary spreader, the second application made perpendicular to the first. Fertilization took place between November 1, 1997, and February 28, 1998.

Two sample trees were chosen per miniplot for detailed growth measurement. These trees were not necessarily adjacent, but were similar in height and diameter. Measurements were made prior to the initial growing season following treatment, following the third growing season, and following the fifth growing season. Diameter, height, and crown length were measured on all sample trees, and local density was measured with a 20 BAF prism. Swiss needle cast intensity was measured with several indices, including foliage color, crown density, foliage transparency, and the number of years of foliage retention.

Four treatment responses were tested, including foliage retention, height growth, basal area growth, and volume growth. Due to the large number of covariates typical of this type of field trial, analysis was performed as a series of regression models, with fertilizer treatments as one set of predictor variables. As the initial phase of analysis, all responses were transformed logarithmically to help accommodate nonlinear relationships and ameliorate expected non-constant variance.

### **Results and Discussion**

#### **Foliage Retention**

Approximately 59% of the variation in the logarithm of year 3 foliage retention was explained by a model containing initial foliage retention, initial crown ratio, and block effects as covariates. The model for the logarithm of year 5 foliage retention, containing the same covariates, explained 41% of the variation. Inclusion of treatment effects in these models indicated that fertilizer treatment did not have a significant effect on foliage retention after either three or five years.

#### **Height growth**

Height growth of the two measure trees within each miniplot was assessed as a response to both fertilization and initial Swiss needle cast severity. Approximately 37% of the variation in the logarithm of threeyear height growth was explained by a model containing initial height, site index, age, and block effects as covariates. The model for the logarithm of five-year height growth, containing initial height and block effects as covariates, explained 29% of the variation. Inclusion of treatment effects in these models indicated that fertilizer treatment did not have a significant effect on height growth after either three or five years.

#### **Basal area growth**

Approximately 72% of the variation in the logarithm of three-year basal area growth was explained by a model containing initial miniplot basal area, local density, average initial crown ratio, and block effects as covariates. The model for the logarithm of five-year basal area growth, containing the same covariates, explained 73% of the variation. Inclusion of treatment effects in these models indicated that fertilizer treatment had a significant effect on basal area growth after both three and five years.

Following three years of treatment, the increase in growth over control plots amounted to 13.8, 9.8, and 16.5 % for N, NPK, and NPKplus treatments, respectively (Fig. 1). The only significant difference between treatments was between the NPK and NPKplus treatments. The growth of NPKplus-treated plots was 6.2% greater than that of NPK treated plots (p=0.0431). Following five years of treatment, the increase in growth over control plots amounted to 11.0, 6.5, and 13.4% for N, NPK, and NPKplus treatments, respectively (Fig. 1). The only significant difference between treated plots was between the NPK and NPKplus treatments. The growth of NPKplus-treated plots was 6.5% greater than that of NPK treated plots (p=0.0221).

#### **Volume growth**

Approximately 91% of the variation in the logarithm of three-year volume growth was explained by a model containing initial miniplot basal area, local density, average initial crown ratio, and block effects as covariates. The model for the logarithm of fiveyear volume growth, containing the same covariates, explained 93% of the variation. Inclusion of treatment effects in these models indicated that fertilizer treatment had a significant effect on volume growth after both three and five years.



Figure 1. Treatment effects as growth increase relative to controls. Bars marked by the same letters are not significantly different ( $\pm$ =0.05).

Following three years of treatment, the increase in growth over control plots amounted to 13.9, 10.9, and 15.6 % for N, NPK, and NPKplus treatments, respectively (Fig. 1). This positive growth effect was not significantly different between treatments.

Following five years of treatment, the increase in growth over control plots amounted to 8.2, 6.1, and 10.2 % for N, NPK, and NPKplus treatments, respectively (Fig. 1). The only marginally significant difference between treated plots was found to be between the NPK and NPKplus treatments. The growth of NPKplustreated plots was 3.9 % greater than that of NPK treated plots (p=0.0774) over five years.

## Conclusions

Fertilization significantly accelerated volume growth, primarily through its effect on basal area growth. Fertlization caused no significant improvement in foliage retention, so did not offer any obvious amelioration of Swiss needle cast by the end of the 5-yr response period.

# *The Effect of Nutritional Status of Douglasfir on* Phaeocryptopus gaeumannii:

# Evidence from foliar chemistry and stable isotopes

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## Introduction

Growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) in the Oregon Coast Range has been significantly impaired by Swiss needle cast (SNC) during the past decade (Hansen *et al.*, 2000, Maguire *et al.*, 2002). The causal agent, *Phaeocryptopus gaeumannii* (Rohde) Petr., is a common ascomycetous endophyte in the natural range of Douglas-fir (Chastagner and Byther, 1984; Tainter and Baker, 1996; Hood, 1997). The length of time required for fungal fruiting bodies (pseudothecia) to emerge from a Douglas-fir needle is highly variable; initial emergence can occur in needles that range from three months to seven years in age.

It is important to understand processes controlling fruiting of pseudothecia because physiological impacts of the disease are coupled to emergence of pseudothecia (Tainter and Baker, 1996), including reduced stomatal conductance and carbon uptake (Manter *et al.*, 2000). Growth productivity losses are linked to losses in carbon gain and premature excision of infected needles, especially in younger needle age classes (Maguire *et al.*, 2002).

Processes by which P. gaeumannii obtains nutrients and the role of nutrient availability in pseudothecia production are not well understood. It is inferred that the fungus acquires nitrogen (N) and carbon (C) from available nutrients in intercellular (apoplastic) spaces of host needle tissues. In support of this inference, Capitano (1999) found no evidence of physical penetration of mesophyll cells when examining infected needles using scanning electron microscopy. Therefore, increases in needle nutrient levels may improve fungal nutrition. Host nutritional balance can alter foliar free amino acid availability availability in the apoplast, with implications for fungal nutrition (Turner and Lambert, 1986; Solomon and Oliver, 2001; Schaberg et al., 2002; Solomon and Oliver, 2002). Major increases in foliar free amino acids are associated with high N availability (N sholm and Ericsson, 1990; Ericsson et al., 1993; Marschner, 1995) and bud break (Van den Driessche and Webber, 1977). Needle turnover also results in the production and translocation of free amino acids out of senescing tissues.

Stable isotope analysis is a useful tool for analyzing host-pathogen nutrient dynamics. Isotopic signatures of fungi associated with plant tissues are influenced by metabolic activities of the fungi as well as by the isotopic ratios of their nutrient source (Gebauer and Taylor, 1999; Henn and Chapela, 2001). Tracing nutrient dynamics between endophytic fungi and their host using stable isotopes may identify forms of assimilated N and C and their sources within the needle. Once these forms and sources of N for *P. gaeumannii* are identified, their dynamics could be examined as the basis for recent intensification of SNC outbreaks.

The objectives of this study are: (1) to examine the relationship between needle N concentration, foliar free amino acids and disease severity in Douglas-fir foliage, and (2) to determine if the N and C isotopic signals can provide insight into the host-pathogen nutrient dynamics. We hypothesize that: (1) increased N availability in Douglas-fir needles will result in increasing numbers of pseudothecia emerging from stomata; (2) isotopic signatures in the fungus will reflect isotopic values in Douglas-fir needles.

#### Methods

#### Study site

The study was conducted during 2001 and 2002 at the USDA-Forest Service Priest River Experimental Forest (PREF) in Priest River, Idaho USA (latitude: 48°22' N, longitude: 116°92' W, altitude: 700 m). Experiments were conducted on ten-year-old Douglas-fir from a progeny-test of low elevation open-pollinated seed sources at PREF. All trees in the experiment were naturally infected with *P. gaeumannii* at an average severity of 0, 0.33 (0.1), 2.3 (0.1) and 3 (0.1) for age classes current, one, two and

three-year-old respectively, using a 0-4 scale, where 0= no pseudothecia, 1=light infection, 2=moderate infection, 3=severe infection. The average needle retention was 2.7 (0.06) years. (Van Aelst-Bouma, unpublished data).

#### **Fertilizer treatments**

In June 2001, post budbreak, two levels of granular urea (46% N,  $\delta^{15}N = 0.28\%$ ) were incorporated into the soil around randomly selected trees. In addition to five control trees, five trees received either a high N treatment (HU = 490 g urea/ tree), or a low N treatment (LU = 40g urea/tree), for a total of 10 trees. This amendment was insufficient to raise foliar nitrogen concentrations, thus a second amendment of 1000g of <sup>15</sup>N-enriched urea ( $\delta^{15}N = 3.69\%$ , Isotec Inc., Champaign, IL) was applied prior to budbreak in April 2002. <sup>15</sup>N tracer was added to the 2002 urea applications to augment the isotopic signature, making it easier to track foliar and fungal uptake.

During May 2002, an additional experiment was implemented to examine the effects of foliar N amendments on five additional sample trees. Miracid (Miracle-Gro<sup>®</sup>, 39%N as ammonia and urea,  $\delta^{15}$ N enriched to 26.37‰ (Isotec Inc.), 15 % C and  $\delta^{13}C = -42$ ) applications were made on three branches located 1.2m above the ground. All age classes of foliage were sprayed with the fertilizer solution, which had been mixed with tap water; untreated control branches were assigned from the whorl above the treated branches on the same tree and were covered with

plastic bags during spraying to prevent contamination.

#### Sample collection

In August 2002 foliar samples from all treated and control trees were collected for FAA quantification and in October 2002 for %N, stable isotope analysis and evaluation of disease severity. Several branches containing all foliar age class (current-year growth to threeyear-old needles) were randomly sampled from each tree at 1.2m. Samples were placed in plastic bags and transported in a cooler to the laboratory at the University of Idaho (Moscow, Idaho) and kept frozen at -20 °C prior to processing.

#### FAA quantification

Randomly selected needles from all age class were used to quantify total FAA following Moore and Stein's ninhydrin reagent method (Moore and Stein, 1954).

Percent nitrogen,  $\delta^{15}N$ , percent carbon and  $\delta^{13}C$  quantification

Quantification of %N,  $\delta^{15}$ N, percent carbon (%C) and  $\delta^{13}$ C in needles and fungal pseudothecia from needles sampled in October 2002, was performed using a continuousflow elemental analyzer (CE Instruments, Italy) coupled to an isotope ratio mass spectrometer (Finnigan, Bremen, Germany). Needles from the foliar Miracid treatment were surface washed in double-deionized water (DDI) before analysis to remove remaining residue of the applied fertilizer and tracer. Pseudothecia were dislodged from stomata of two- and three-year-old needles by suction; there was not enough pseudothecia in one-yearold needles. Detached pseudothecia were collected in DDI and freeze dried (Labconco/Freezone 4.5, Kansas City, MO). The vacuumed twoand three-year-old needles and randomly sampled needles from the current and one-year-old age classes were oven dried at 65°C and ground to a fine homogeneous powder for the analysis. Needles and associated pseudothecia were pooled by needle age class across all sample branches within individual trees.

#### **SNC** Severity

To quantify the effect of N on the severity of Swiss needle cast, pseudothecia density was estimated in accordance with Hansen *et al.* (2000). One hundred adjacent stomata per needle per needle age class were examined at 50x magnification and the number of observed pseudothecia was noted from ten randomly chosen needles per age class per tree.

#### N uptake by P. gaeumannii

The following mixing model was employed to identify source N taken up by *P. gaeumannii*:

 $X_{tracer} = (\delta_{sample} - - \delta_{background}) / (\delta_{tracer} - - \delta_{background}) \qquad eq. 1$ 

sponse.

Where (X <sub>tracer</sub>) is the fraction of tracer N in the sample. Multiplying (X <sub>tracer</sub>) by 100 gives the percentage of N in the sample from the tracer.  $\delta_{background}$  is the value in the control samples.

slopes of the trend line for each individual tree in the experiment. Slopes were compared to each other in an ANOVA analysis followed by a least squares mean separation using Tukey's adjustment.

#### Results

**Statistical analysis** 

Experiments were conducted in

a split-plot design, where N fertiliza-

tion served as the plot, individual

trees served as blocks and needles

were observational units. Analyses

of the data collected could not be

performed in an analysis of variance

(ANOVA) framework because of the

violation of the assumption of in-

dependence of variables, i.e. needle

age. Needles get infected soon after

emergence; therefore, older needles

acquire the P. gaeumannii inoculum

at age zero (budbreak). The two

models used to investigate the ef-

fects of fertilizers consisted of a com-

parison of residuals and an order-

one autoregressive structure. All sta-

tistical analyses were performed in

SAS release 8.2 (SAS Institute, Carv,

NC) using PROC Mixed, Akaike's

information criterion, and Tukey's

adjustment for least squares mean

separations. Where interactions be-

tween factors were significant,

ANOVA was performed within each

age class to assess differences in re-

and  $\delta^{15}N$  in pseudothecia was inves-

tigated using a comparison of the

The relationship between %N

#### **Responses to soil nitrogen treatments**

#### Needle nitrogen and carbon

Nitrogen application to the soil increased foliar %N in HU treatments relative to controls in all needle age classes. The LU treatment was significantly higher in % N relative to the control in two- and three-yearold needles only (Table 1, Fig. 1a). In the HU treatment the increased  $\delta^{15}$ N in all needle ages relative to the CK treatments, especially current-yearneedles, reflected uptake of N fertilizer (Table 1, Fig. 1b).

LU significantly increased %C of Douglas-fir needles in some needle age classes (Table 2, Fig. 1c); however, <sup>13</sup>C levels remained unaffected by soil N treatments (Table 2, Fig.1d).

#### Foliar free amino acids

FAA concentration increased with N fertilization treatment in current-year-needles only (Table 1, Fig. 2a).

#### Disease severity

Soil N treatments increased disease severity in needle ages two and three (Table 1, Fig. 2b). In two-yearold needles infection was 2.2 and 3.6 fold higher in HU and LU respectively than in CK (13 and 21% of stomata occluded versus 6%). In three-year-old needles infection was 2.3 and 2.4 fold higher in HU and LU respectively than in CK (18 and 18% of stomata occluded versus 8%) (Fig. 2b).

#### Pseudothecia nitrogen and carbon

Pseudothecia %N was higher than %N in the source needles in



Figure 1. Current, one-, two-, and three-year-old Douglas-fir needles sampled in October 2002 following different levels of soil N applications: control, low urea, and high urea; 1a: Percent N (%N), 1b: Nitrogen stable isotope ratio ( $\delta^{15}$ N), 1c: Percent carbon (%C), and 1d: Carbon stable isotope ratio ( $\delta^{13}$ C). Stars indicate significantly different values. Bars represent standard error. P=0.001 and n=5.

two- and three-year-needle age classes (Figure 3a, 1a). Nitrogen applications increased %N in pseudothecia emerging from twoand three-year-old needles. Pseudothecia had significantly lower  $\delta^{15}N$  relative to the needles in all treatments and were depleted in <sup>15</sup>N in fertilized needles compared to controls (Fig. 3b). Pseudothecia emerging from two- and three-yearold needles in trees receiving the LU treatment were significantly depleted in <sup>13</sup>C compared to pseudothecia emerging from control needles, while pseudothecia emerging from HU needles had lower  $\delta^{13}C$  in threeyear-old needles (Fig. 3d).

Percent N in needles and pseudothecia was positively corre-



Figure 2. Current, one-, two-, and threeyear-old Douglas-fir needles sampled in October and August 2002, respectively, following different levels of soil N applications: control, low urea, and high urea. 2a. Free amino acids (FAA), 2b. Disease severity (percent of stomata clogged with pseudothecia). Stars indicate significantly different values. Bars represent standard error. P= 0.001 and n=5.



Figure 3. Pseudothecia associated with two- and three-year-old Douglas-fir needles sampled in October 2002 following different levels of soil N applications: control, low urea, and high urea; 3a: %N, 3b:  $\delta$ 15N, 3c: %C, and 3d:  $\delta$ 13C. Stars indicate significantly different values. Bars represent standard error. P=0.001 and n=5.

			Soil treatm	Foliar treatment response variables						
Predictor variables		Needles			Pseudo	othecia	Ne	edles	Pseudothecia	
	%N (%/g DW)	δ <sup>15</sup> N [ 0]	FAAµmol/g DW	Severit y(%)	%N (%/g DW)	δ <sup>15</sup> N[ 0] [ 0]	%N (%/g DW)	δ¹5N [ 0]	NC (%/g DW)	δ <sup>15</sup> N [ 0]
Treatment	6.98**	32.85**	12.35**	15.69**	260.89**	189.25**	0.96	21.02**	833.66**	884.74**
Age	6.03**	9.65**	16.87**	82.27**	16.26**	340.69**	<b>3</b> .5 <sup>*</sup>	8.64**	96.05**	216.08**
Treatment x Age	2.16	17.19**	3.93**	7.67**	39.38**	42.52**	0.45	<b>3.20</b> *	143.20**	22.75**

Table 1. F ratios for the respective tests. Significance of ratios is footnoted. Responses to soil and foliar fertilization were analyzed as separate experiments with separate controls

\*P< 0.05, \*\*P< 0.01, n= 5

Table 2. F ratios for the respective tests. Significance of ratios is footnoted. Responses to soil and foliar fertilization were analyzed as separate experiments with separate controls

Predictor variables	Soi	treatment i	response varial	oles	Foliar treatment response variables					
	Needles		Pseudothecia		Nee	lles	Pseudothecia			
	% <b>C</b>	δ <sup>13</sup> C	% <b>C</b>	δ <sup>13</sup> C	% <b>C</b>	δ <sup>13</sup> C	%C	δ <sup>13</sup> C		
	(%/g DW)	[0]	(%/g DW)	[0]	(%/g DW)	[0]	(%/g DW)	[0]		
Treatment	<b>4.45</b> *	0.01	98.03 <sup>**</sup>	1095.35**	1.07	0.01	71.72**	93.16**		
Age	4.40**	5.77**	20.77**	11.76**	0.73	6.37**	62.76**	55.44**		
Treatment <sup>*</sup> Age	0.49	1.46	2.51	7.59**	0.98	1.85	91.99**	31.65**		

\**P*< 0.05, \*\**P*< 0.01, *n*= 5

lated when averaged across all treatment and control trees ( $r^2 = 0.60$ , Fig. 4). However, the level of N in the foliage was far more variable relative to % N in the pseudothecia. Percent N and  $\delta^{15}$ N in pseudothecia was negatively correlated (Fig. 5). The HU treatment had a significantly different slope from LU and controls consistent with uptake of N enriched in <sup>15</sup>N.

#### **Responses to foliar nitrogen treatment**

#### Foliage nitrogen and carbon

Miracid-treated needles were not significantly different in %N relative to the non-sprayed controls (Table 1, Fig. 6a). However, foliar  $\delta^{15}$ N was significantly higher in Miracid-treated

needles in age classes two and three compared to non-sprayed controls (Fig. 6b).

Pseudothecia nitrogen and carbon

Pseudothecial %N increased following Miracid applications in twoyear-old needles compared to the unsprayed controls, and no difference was observed in three-year-old needles (Fig. 7a). Pseudothecia from Miracid-treated needles were enriched in  $\delta^{15}$ N relative to controls (Table 1, Fig. 7b).

#### C: N ratio in pseudothecia

Percent N and C in pseudothecia were positively correlated across all treatments ( $r^2 = 0.82$ , Fig. 8). The C: N ratio of approximately 15 is highly conserved across a range of %N and %C values.

#### Discussion

The fertilizer response varied with needle age and treatment level. Increased %N in all treated needle age classes with the exception of LU current needles indicated uptake of fertilizer. Current-year needles of LU showed poor N response likely because of late application (i.e. postbudbreak in 2001) and relatively low initial application rates. In addition, a partial immobilization of N amendments by soil microorganisms and soil particles may have limited available N in the LU treatment (Kaye and Hart, 1997; Magill et al., 1997). Interestingly, an increased level of N



Figure 4. Percent nitrogen in two- and three-year-old Douglas-fir needles and associated pseudothecia sampled in October 2002 following different levels of soil fertilizer applications. HU = High urea for two-year-old needles, HU3 = High urea for three-yearold needles, LU2 = Low urea for two-year-old needles, LU3= Low urea for three-year-old needles, CK2= Control for two-year-old needles, and CK3= Control for three-year-old needles. Points represent means. Bars represent standard error. P=0.001. n=5.

Figure.5.  $\delta$ 15N versus percent nitrogen in pseudothecia from Douglas-fir foliage receiving different soil nitrogen treatments and the control. Points represent pseudothecia emerging from two-, and three-year-old Douglas-fir needles sampled in October 2002 following different levels of soil fertilizer application: control, low urea, and high urea. Lines represent slopes. n=1.



Fig.6: Current, one-, two-, and three-year-old Douglas-fir needles sampled in October 2002 following foliar N application: Miracid, and Miracid control; 6a: %N, 6b:  $\delta$ 15N, 6c: %C, and 6d:  $\delta$ 13C. Stars indicate significantly different values. Bars represent standard error. P=0.001 and n=5.



Figure 7. Pseudothecia associated with two- and three-year-old Douglas-fir needles sampled in October 2002 following foliar N application: Miracid, and Miracid control ; 7a: %N, 7b:  $\delta$ 15N, 7c: %C, and 7d:  $\delta$ 13C.

Stars indicate significantly different values. Bars represent standard error. P=0.001 and n=5.



Figure 8. Percent carbon versus percent nitrogen in pseudothecia, associated with two and three-year-old Douglas-fir foliage, sampled in October 2002 following different levels of soil and foliar N treatments: High urea for two-year-old pseudothecia, High urea for three-year-old pseudothecia, Low urea for two-year-old pseudothecia, Low urea for three-year-old pseudothecia, Control for twoyear-old pseudothecia, and Control for three-year-old pseudothecia, MA-CK two-year-old needles, MA for twoyear-old pseudothecia, MA-CK three-year-old pseudothecia, and MA three-year-old pseudothecia. P=0.001. n=1. y = 10.394x + 8.5989R2 = 0.8165

applied in 2002 prior to budbreak still did not stimulate a response in foliar %N in current-year-needles of the LU treatment. This lack of response is evident in both %N and  $\delta^{15}$ N in LU current-year-needles. Millard and Proe (1992) observed a similar lag in fertilizer uptake in *Picea sitchensis* in the first year of fertilization.

Based on analysis of the %N and  $\delta^{15}N$  in the two- and three-year-old needle age classes it appears that elevated %N in LU and HU resulted from different mechanisms. In the HU trees, increased %N in older foliage reflects soil N uptake as indicated by the elevated  $\delta^{15}N$  of needles. However, in LU treatments the higher levels of %N were not associated with changes in foliar  $\delta^{15}N$  indicating the higher levels are not from uptake but perhaps from microbial

N or internal sources. In both cases the increase in foliar N in two- and three-year-old needles resulted in increasing development of pseudothecia.

The  $\delta^{15}$ N values of *P. gaeumannii* were 2 to 3‰ lower (depleted in  ${}^{15}N$ ) as compared to  $\delta^{15}N$  in associated Douglas-fir needles. This contrasts to N isotopic ratios from most other fungal taxonomic groups that are isotopically enriched (more <sup>15</sup>N) in comparison to the hosts they are associated with (Hobbie et al., 1999; Henn and Chapela, 2001). It has been hypothesized that biosynthetic processes will lead to the production of amino acids that are isotopically depleted relative to source N (Macko et al., 1986; Hobbie et al., 1999). Thus, as %N in pseudothecia follows the %N increase in fertilized needles (Fig. 3b), P. gaeumannii takes up more N (presumably as amino acids) becoming isotopically depleted in <sup>15</sup>N relative to host N (Fig. 5). However, it should be noted that the <sup>15</sup>N depletion in pseudothecia can be caused by a combination of: (1) uptake of a depleted N source (amino acids) within the needle, (2) fractionation of N upon uptake, or (3) fractionation of N due to biosynthetic processes within the fungus.

In addition, considerable differences in <sup>15</sup>N levels between the foliar applied enriched N and fungal pseudothecia ( $\delta^{15}N = 3.69\%$  and 0‰ respectively) indicate that P. gaeumannii hyphae acquired a portion of the N from the Miracid applied to the needle surface and a portion from the bulk N pool within the needle. The mixing model results indicate that a higher percentage of the fungal N was from the foliar applied N relative to the bulk needle N thus indicating the possibility that the fungus can acquire external N through its hyphae.

*P. gaeumannii* pseudothecia from both Miracid-treated and control needles exhibited  $\delta^{13}$ C levels comparable to associated needles (around -27‰), while  $\delta^{13}$ C of Miracid was measured at around -42‰. If *P. gaeumannii* were to acquire C from the Miracid applied to needle surfaces, a further depletion in <sup>13</sup>C would be measured. This finding implies that fungal hyphae most likely acquired C from intercellular spaces in the needle or the form of C in Miracid is not available to the fungus.

Percent N and C in pseudothecia were positively correlated ( $r^2 = 0.82$ , Fig. 8), indicating that the processes of acquiring both elements by fungal hyphae are closely related.

To our knowledge this is the first report describing the N and C isotopic relationship between an endophytic fungus and its host. Previous fungi-host studies have focused primarily on mycorrhizae (Hobbie *et al.*, 1999; Emerton *et al.*, 2001) and saprophytes (Gebauer and Taylor, 1999; Henn and Chapela, 2001). This study measured increased fungal fruiting associated with increased N uptake from host tissue, limited increase in  $\delta^{13}$ C, and a decrease in –  $\delta^{15}N$  in pseudothecia of *P. gaeu*mannii in fertilized Douglas-fir needles. These results are consistent with the uptake of simple nitrogenous compounds, and perhaps other compounds present in intercellular spaces of needles, such as sucrose (Gleixner et al., 1998) or fractionated starch (Scott et al., 1999). An explanation for the cause of the current SNC outbreak in the PNW remains equivocal, but may be associated with increased N availability to Douglas-fir trees or to other environmental factors that favor fungal metabolism relative to the host.

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