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Assessment of Swiss Needle Cast Disease: Temporal and Spatial Investigations of Fungal Colonization and Symptom Severity

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Abstract

Increasing severity of Swiss needle cast (SNC), a foliar disease of Douglas-fir caused by the fungus *Phaeocryptopus gaeumannii*, has become a matter of concern in forest plantations throughout coastal Oregon and Washington. This study monitored SNC disease in three Oregon Douglas-fir plantations bi-annually in 1998–1999, and compared differences in fungal colonization and symptom development in trees from north- and south-facing plots at each plantation. Fungal colonization as quantified by ergosterol content, pseudothecia density and quantitative PCR was significantly correlated with symptom severity (needle retention and needle cholorosis). All three measures of fungal colonization were highly correlated with each other; and only the ergosterol–pseudothecia relationship differed between plots, presumably due to the non-species specific nature of ergosterol measurements. Differences in symptom severity and fungal colonization between north- and south-aspect plots were consistent with climate differences. At low to moderate levels of infection, trees growing on warmer (i.e. south slopes in the western, and north slopes in the eastern Coast Range) slopes had higher levels of colonization, particularly during the winter months. Plots with southern exposures, which received greater amounts of solar radiation, had greater amounts of needle abscission compared to north-aspect plots with similar amounts of fungal colonization. As a result, greater fungal abundance and symptom expression developed on south-aspect slopes within the Oregon Coast Range.

Introduction

Swiss needle cast (SNC), caused by the fungus *Phaeocryptopus gaeumannii* (Rhode) Petrak, is a foliar disease of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). *Phaeocryptopus gaeumannii* occurs throughout the range of Douglas-fir, and has been considered a relatively minor pathogen in forest plantations (Hansen et al., 2000). However, since the mid-1980s, forest managers and researchers have noticed an increase in frequency and severity of SNC in coastal plantations in Oregon and Washington. Aerial surveys conducted by the Oregon Department of Forestry in 1999 detected *c*. 119 500 ha of plantations with visible chlorosis and premature needle loss (Hansen et al., 2000), and extensive growth losses. For example, based on a 1996 survey, Maguire et al. (2002) estimated that on 52 611 ha of forested lands in western Oregon, SNC caused an *c*. 23% reduction in volume growth, or an implied growth loss of 3.2 m^3/ha/year.

Several methods for the quantification of *P. gaeumannii* colonization are currently available – ergosterol content, quantitative PCR, and presence of fruiting bodies or pseudothecia density (Manter et al., 2001; Winton et al., 2003). Spring measurements of all quantification techniques are significantly correlated with each other (Hansen et al., 2000; Manter et al., 2001; Winton et al., 2003); however, it is unknown whether the timing of sample collection may influence the correlations. For example, it is possible that maximum fungal colonization may not coincide with maximum symptom expression. Furthermore, only one of these studies has examined site-specific relationships (Manter et al., 2001) between the quantification techniques, and found that the relationship between fungal biomass (estimated by ergosterol content) and fungal fruiting (pseudothecia density) varied by site. Because of the non-species specific nature of the ergosterol (a membrane sterol found in nearly all higher fungi) assay, this variation may be due to the presence of non-target

fungi growing in the phyllosphere, and/or differential expression of sporulation (i.e. greater sporulation per unit biomass). In regard to the latter, sporulation in some species of fungi is influenced by temperature, light, etc. (Griffin, 1994), and because these factors vary spatially (i.e. due to topography), the relationships between the different fungal colonization measures may vary with plot aspect. Knowledge of such relationships is paramount to selecting the most appropriate measure of fungal colonization to accurately describe disease pressure, as the pathogenicity of *P. gaeumannii* has been related to the amount of pseudothecia and stomatal occlusion (Manter et al., 2000), not necessarily total pathogen biomass.

Previous studies have also reported correlations between fungal colonization and symptom severity (i.e. needle chlorosis and retention). However, spatial variation in symptom expression is relatively high in forest plantations (e.g. topographical and within tree canopies, Hansen et al., 2000). Whether this variation is due to spatial differences in fungal colonization or symptom expression is not known.

As our knowledge regarding the observed spatial heterogeneity of symptom severity and fungal colonization is limited, the main objectives of this study were to: (i) quantify the seasonal patterns of *P. gaeumannii* colonization and symptom severity in three stands with various levels of SNC, (ii) evaluate the relationship between fungal colonization measures and symptom severity on north and south-facing plots, and (iii) evaluate the relationship between fungal colonization measures and symptom severity within tree canopies.

Materials and Methods

Sample sites

Three 12–15-year-old Douglas-fir plantations with varying levels of SNC were chosen for study. Paired permanent plots were created on north- and south-aspect slopes (10–30%) at each site. Each permanent plot consisted of a group of six infected trees and six uninfected controls, which were created for concurrent physiology studies being conducted on these sites. In addition, inclusion of fungicide-sprayed trees in this study also helped to increase the power of regression analysis by increasing the range of fungal colonization levels within in each plot. Chlorothalonil (Bravo 720, rate = 66 ml per 3.78 l, applied until run-off) by means of a backpack sprayer was applied to control trees in 1998–1999 at bud break (90% trees had broken bud) and 1 month following bud break. The high disease site was located on the Siuslaw National Forest, near Beaver, OR, USA [Beaver north (BN) and south (BS) plots, respectively], the medium disease site was located on the Siuslaw National Forest near Hebo, OR, USA [Hebo north (HN) and south (HS) plots, respectively], and the low disease site was located on the MacDonald-Dunn Forest near Corvallis, OR, USA [Mac north (MN) and south (MS) plots, respectively].

Tree sampling

At each sample date two primary lateral branches from each tree were removed, placed in plastic bags, and transported back to the laboratory on ice in cooler boxes. Once in the laboratory, needles from each sampled tree were pooled by needle cohort (i.e. year of initiation) and stored at 0°C. All measures of fungal colonization and symptom severity were determined on subsamples from the pooled samples within 1 month of collection. All analyses from these trees were limited to branches from the south side of the lower half of the tree canopy. Ergosterol content, pseudothecia density, needle retention and chlorosis measurements were conducted on branches harvested in July 1998, December 1998, May 1999 and November 1999. Quantitative PCR measurements were conducted on branches harvested in May 1999 and November 1999.

Canopy position sampling

Three infected trees from each of five SNC affected plots were sampled in February 2000. Plots were the BN, BS, HN, HS stands described above, and an additional plot at Sour-grass Summit, Grande Ronde, OR, USA. One branch from each of the north-top, north-bottom, south-top and south-bottom quadrants of each tree was sampled and analysed for fungal colonization and symptom severity.

Fungal and symptom quantification

Ergosterol content (μg ergosterol/g needle dry weight), quantitative PCR (picogram *P. gaeumannii* DNA per nanogram *P. menziesii* DNA), and pseudothecia density (percentage of stomata with visible pseudothecia) were determined according to the methods outlined in Manter et al. (2001) and Winton et al. (2002). All assays were measured on randomly selected subsamples of *c.* 25, 10 and 10 needles, respectively, from the needle collections described above. Needle retention was visually estimated to the nearest 10% for each needle cohort (internode), and needle chlorosis was rated on a scale of 0–3, with 0 being dark green, one slight, two moderate and three severe chlorosis.

Weather data

At each site, air temperature (°C), relative humidity (RH, %), and incident photosynthetic photon flux density (PPFD, μmol/m^2/s) were recorded hourly with portable dataloggers (Spectrum Technologies, Inc., Plainfield, IL, USA). Temperature/RH dataloggers were protected from direct radiation by a radiation shield (Spectrum Technologies, Inc.) and placed in an open area (*c.* 5 m radius) at *c.* 1.5 m above the ground. As only one datalogger was installed at each plot, equipment biases were removed by random reassignment of dataloggers every month.

Analysis

All graphical presentations depict the arithmetic mean and individual standard error for the six trees sampled

at each plot (e.g. BN, BS, etc.). Differences in fungal colonization and symptom development between plot-aspect (north and south) and fungicide-application (sprayed and unsprayed) groups were tested using the repeated measures analyses described by Looney and Stanley (1989). Due to the limited sampling on a tree-basis (e.g. 10–25 needles per tree), all correlation analyses were determined using the arithmetic mean for the six trees per group at each plot. The influence of all treatment factors (i.e. date, site, slope, fungicide-spray and needle age) on the relationships between the various independent variables was analysed by dummy-variable regression (Steel et al., 1997), only the significant factors ($P < 0.05$) are presented. Canopy position data were analysed as a randomized complete block with PROC MIXED (Vers. 8.1, SAS Institute Inc., Cary, USA), where canopy position was a fixed effect and tree and site attributes were random effects.

Results

Phaeocryptopus gaeumannii was present in all plot trees sampled. In unsprayed trees, pseudothecia density varied with needle cohort, site, and sampling date (Fig. 1). The application of chlorothalonil successfully limited colonization in all needle cohorts assayed (cf. Fig. 1 and Table 1). The most effective control was achieved on the newly expanding needles (i.e. 1998 cohort), and at the end of this study no pseudothecia were observed in these needles. Pseudothecia density also was significantly reduced, but to a lesser extent, in the fully expanded, older needles (i.e. 1997 and 1996 cohorts) that were infected prior to the first fungicide-application.

Overall, all three measures of fungal colonization (i.e. pseudothecia density, ergosterol content and quantitative PCR) were significantly correlated with each other, and the measures of symptom severity (i.e. needle retention and chlorosis) (Table 2); however, some

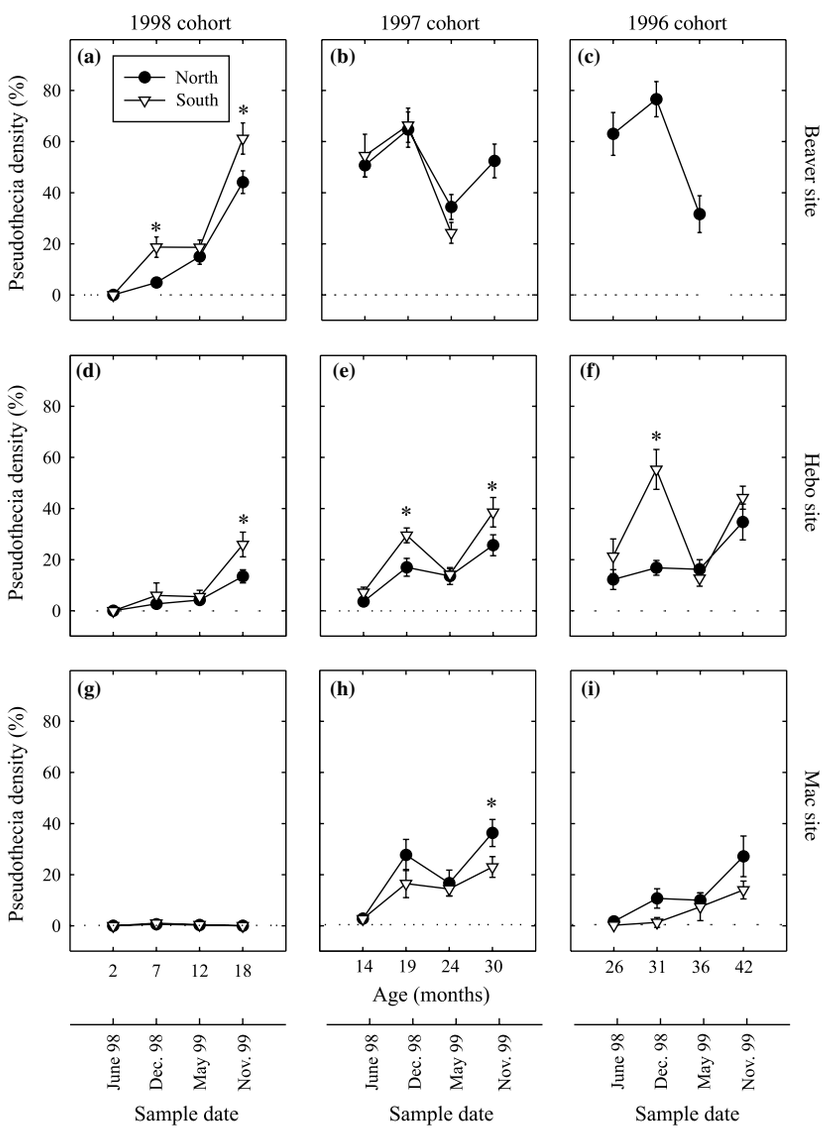

Fig. 1 Mean pseudothecia density of *Phaeocryptopus gaeumannii* over time for unsprayed Douglas-fir trees growing on north- and south-aspect plots at three sites. Each point is the arithmetic mean and SE of six trees. Missing observations were due to needle abscission. Asterisk denotes significant difference between north- and south-aspect plots

Table 1
Difference in *Phaeocryptopus gaeumannii* fungal colonization (i.e. pseudothecia density) between unsprayed and sprayed (chlorothalonil) Douglas-fir foliage

	1998 Cohort				1997 Cohort				1996 Cohort			
	June 1998	December 1999	May 1999	November 1999	June 1998	December 1999	May 1999	November 1999	June 1998	December 1999	May 1999	November 1999
BN	0.0	−4.7	**−14.8**	**−33.1**	**−11.5**	**−21.5**	−6.6	**−19.4**	**−21.7**	**−22.8**	−4.1	–
BS	0.0	**−18.6**	**−18.6**	**−61.2**	**−10.3**	**−18.9**	4.7	–	–	–	–	–
HN	0.0	−2.4	−4.2	**−13.5**	0.4	**−10.2**	**−7.3**	**−17.3**	**−7.7**	**−13.6**	−5.5	**−21.7**
HS	0.0	−5.5	−5.3	**−25.9**	−2.5	**−14.5**	−4.3	**−22.3**	−2.3	**−19.5**	**−7.5**	**−16.0**
MN	0.0	−0.2	−0.3	0.0	3.6	**−18.0**	**−7.9**	−2.1	−0.8	−6.2	−2.7	**−13.2**
MS	0.0	−1.0	−0.4	0.0	1.1	**−13.1**	**−10.0**	−3.3	0.7	0.9	−4.9	**−11.6**

Negative values are reductions due to spraying, and significant values are in bold ($P < 0.05$).

Table 2
Pearson correlation coefficients between *Phaeocryptopus gaeumannii* fungal colonization (i.e. pseudothecia density, ergosterol content and quantitative PCR) and symptom severity (i.e. needle retention and needle chlorosis). All correlations are significant at $P \leq 0.05$

	Pseudothecia density	Ergosterol content	Quantitative PCR	Needle retention
Pseudothecia density	–	–	–	–
Ergosterol content	0.885	–	–	–
Quantitative PCR	0.814	0.733	–	–
Needle retention	−0.644	−0.578	−0.451	–
Needle chlorosis	0.725	0.628	0.677	−0.750

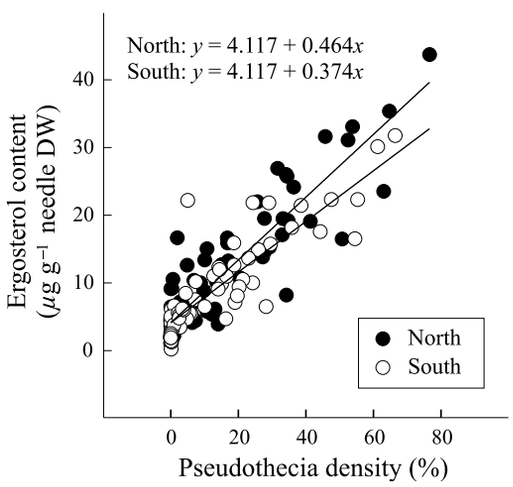

Fig. 2 Relationship between ergosterol content and pseudothecia density for Douglas-fir trees growing on north- and south-aspect plots at three sites. Each point is the arithmetic mean and SE of six trees

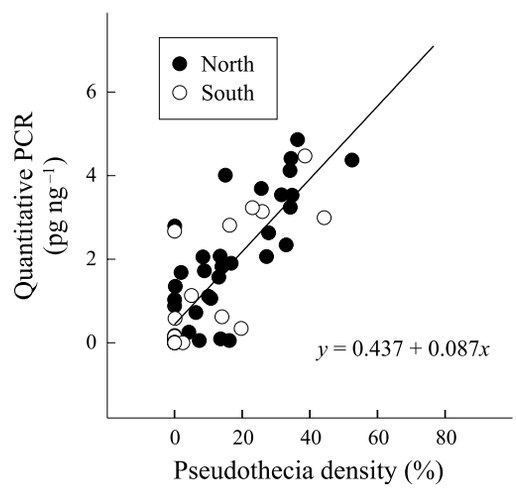

Fig. 3 Relationship between quantitative PCR content and pseudothecia density for Douglas-fir trees growing on north- and south-aspect plots at three sites. Each point is the arithmetic mean and SE of six trees

of these relationships were influenced by sample dates and plot. For example, the relationship between pseudothecia density and ergosterol (Fig. 2), but not quantitative PCR (Fig. 3), differed between north- and south-aspect plots.

The level of fungal colonization, based on all quantitative measures, was consistently the highest at the Beaver site, followed by the Hebo and Mac sites, respectively. For example, pseudothecia densities were *c*. 50, 20 and 5% (Fig. 1), and ergosterol contents were *c*. 28, 12 and 8 μg/mg needle dry weight (data not shown), for the 18-month-old 1998 needle cohort at the Beaver, Hebo and Mac sites, respectively. In general, fungal colonization increased over time, with the largest increases during the late summer/early fall, except during the spring sample (May 1999) when abscission of the most heavily infected needles occurred (cf. Figs 1 and 4). Symptom severity also increased over time (i.e. decreasing needle retention), however, the largest increases occurred during the late winter/early spring (Fig. 4). Due to the delay between fungal colonization and symptom severity, the correlation between winter fungal colonization (December 1998 sample date) and spring symptom severity (May

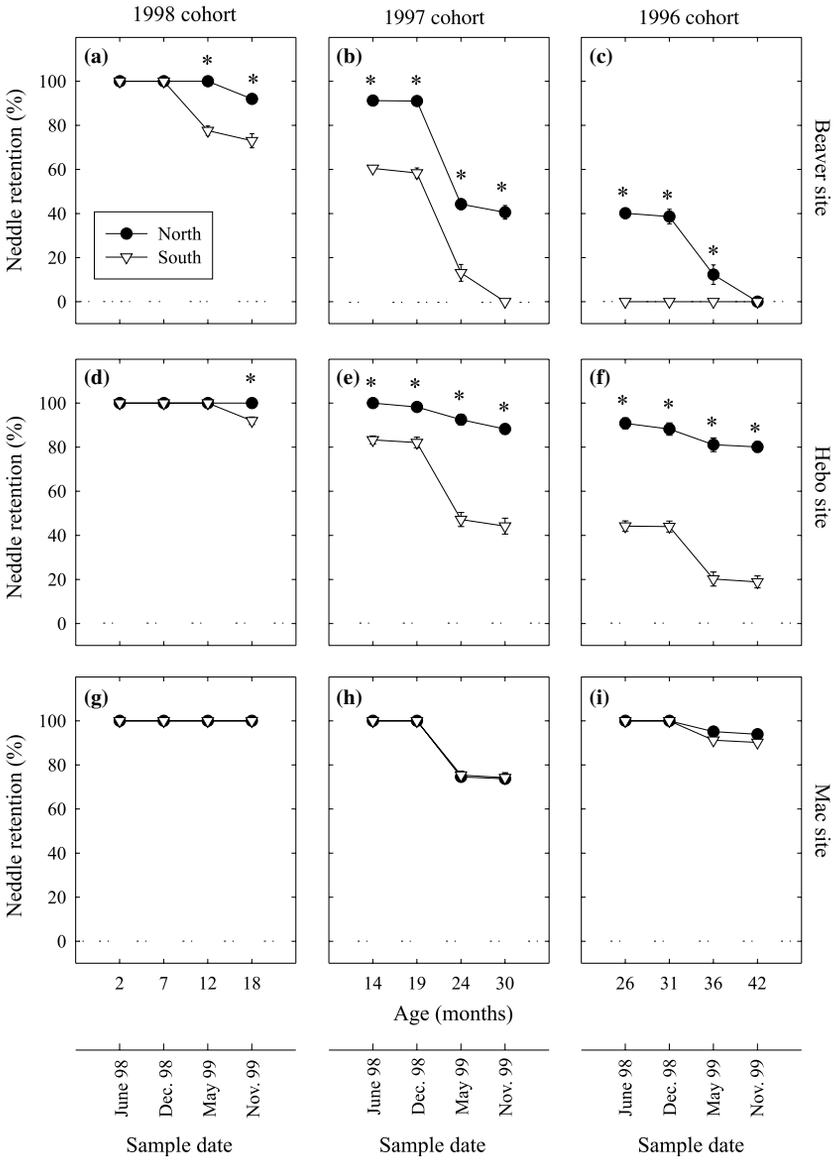

Fig. 4 Mean needle retention over time for unsprayed Douglas-fir trees growing on north- and south-aspect plots at three sites. Each point is the arithmetic mean and standard error of six trees. Asterisk denotes significant difference between north- and south-aspect plots

Table 3
Pearson correlation coefficient between *Phaeocryptopus gaeumannii* colonization (i.e. pseudothecia density, ergosterol content, and quantitative PCR) sampled in December 1998 and symptom severity (i.e. needle retention and needle chlorosis) sampled in May 1999. All correlations are significant at $P \leq 0.05$. Quantitative PCR was not measured for the December 1998 sample

	Pseudothecia density	Ergosterol content	Quantitative PCR
Needle retention	−0.902	−0.816	–
Needle chlorosis	0.810	0.756	–

Table 4
Average mean-daily weather data for north- and south-aspect plots at the Beaver, Hebo and Mac sites, collected hourly from 1 June 1998 to 31 May 1999. PPFD is photosynthetic photon flux density

	Temperature (°C)		PPFD (μmol/m^2/s)		RH (%)	
	North	South	North	South	North	South
Beaver	9.70	10.15	292.8	338.7	86.2	86.3
Hebo	8.58	8.93	275.0	315.8	87.5	88.2
Mac	10.98	10.58	300.4	364.5	81.5	81.2

1999 sample date) was also determined. In this case, the correlations between fungal colonization measures and symptom severity were improved (Table 3).

The weather data collected from each plot is shown in Table 4. For all three sites, PPFD was greater on south plots, whereas temperature differences between plots varied with site. For example, at the two coastal sites, the south plots experienced higher daily mean temperatures, but at the interior MacDonald Forest site, the north plot had higher daily mean temperatures than its south counterpart. The different pattern in temperature regime at the interior site was consistent with the observed daily maximum and minimum temperatures (data not shown). At the interior site the south slope consistently reached a higher daily maximum temperature, like its coastal counterparts.

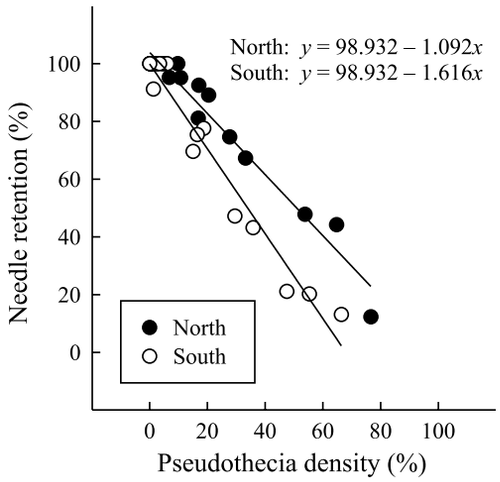

Fig. 5 Relationship between needle retention (May 1999 sample) and pseudothecia density (December 1998 sample) for Douglas-fir trees growing on north- and south-aspect plots at three sites. Each point is the arithmetic mean and SE of six trees

Table 5
Differences in *Phaeocryptopus gaeumannii* fungal colonization (i.e. pseudothecia density, ergosterol content and quantitative PCR) and symptom severity (i.e. needle retention and needle chlorosis) at four canopy positions in Douglas-fir trees. Three trees from each of the Beaver north, Beaver south, Hebo north, Hebo south and Sour-grass Summit plots were sampled

	North		South	
Measure	Top	Bottom	Top	Bottom
Pseudothecia density	12.6 (3.5)^{a}	4.0 (0.9)^{c}	17.5 (4.5)^{a}	6.9 (1.5)^{b}
Quantitative PCR	0.8 (0.2)^{a}	0.3 (0.1)^{b}	0.8 (0.2)^{a}	0.6 (0.1)^{a}
Needle retention	86.7 (4.1)^{b}	98.3 (1.7)^{c}	76.7 (3.4)^{a}	91.7 (3.1)^{b}
Needle chlorosis	0.9 (0.2)^{b}	0.5 (0.1)^{c}	1.5 (0.2)^{a}	1.2 (0.2)^{b}

Mean values with different letters are significantly different at $P \leq 0.05$. Standard errors are in parentheses.

However, its daily minimum temperature was consistently lower, unlike its coastal counterparts, resulting in a lower average daily temperature.

Differences in pseudothecia density were also observed between the north and south plots within each site for the winter samples (i.e. December 1998 and November 1999) (Fig. 1). Differences in pseudothecia density paralleled the mean daily temperature patterns for sites. For example, pseudothecia density was significantly higher for the south compared with the north plots at the coastal sites, but the opposite trend was seen for the interior site. The differences in pseudothecia density between plots varied depending on the needle cohort and age and ranged from 0 to 40 percentage points (Fig. 1). North and south plots also differed in the amount of needles retained for a given level of fungal colonization. Needle retention was lower for the south plots at a given level of pseudothecia density at all three sites (Fig. 5).

The distribution of both fungal colonization and symptom severity within tree canopies was not uniform (Table 5). For example, pseudothecia density was *c*. 10 percentage points higher in the upper portion of the canopy, and *c*. 4 percentage points greater on the south side of the tree. A similar pattern was observed for symptom severity, which also was greatest in the upper canopy and on the south side of the tree. Needle retention was reduced by *c*. 15 percentage points in the upper canopy, and reduced by *c*. 10 percentage points on the south side of the tree.

Discussion

Phaeocryptopus gaeumannii is a widely distributed and very common parasite of Douglas-fir foliage in western North America. Although it is known to cause severe disease problems where Douglas-fir is grown as an exotic species, it has seldom been considered an important pathogen in forest plantations in areas where Douglas-fir is native (Boyce, 1940). The underlying causes for the current SNC epidemic affecting plantations in coastal Oregon and Washington remain unclear and the causal role of *P. gaeumannii* has been questioned (Hansen et al., 2000). In considering the paradox of how *P. gaeumannii* could be widespread, but harmless within the native range of Douglas-fir and still cause catastrophic defoliation in plantations in Europe, Boyce (1940) considered several alternatives and reasoned that differences in climate favouring the growth and reproduction of *P. gaeumannii* were the most likely explanation. In particular, Boyce (1940) contrasted the warm, dry summers of western North America to the humid summers with episodic rain common in Europe. Our results suggest that relatively slight differences in microclimate can influence the colonization of foliage by *P. gaeumannii* as well as the severity of disease symptoms.

The pathogenicity of *P. gaeumannii*, and its role as the causal agent of SNC is supported by the findings in this study. In all of the sites studied here, which exhibit typical SNC symptoms, symptom severity was strongly correlated with the amount of *P. gaeumannii* colonization, particularly pseudothecia density. Furthermore, removal of the fungus by fungicide applications consistently reduced symptom severity.

Fungal colonization of needles was also observed to increase more rapidly on south plots from the coastal sites and the north plot from the interior site, especially during the cooler winter months. The only exception to this pattern occurred in the 1997 cohort of needles at the Beaver site, presumably due to very high levels of colonization in both the north- and south- aspect plot foliage and severe winter storms that caused needle abscission to occur earlier than normal. Abscission of the most heavily colonized needles likely resulted in less colonized needles remaining attached at the time of sampling, causing colonization measurements in the south aspect plot to be lower. Of the weather variables measured here, average daily temperature appears to be the most important factor influencing fungal colonization, as it was the only climate factor to parallel fungal colonization (i.e. BS > BN, HS > HN and MN > MS). Previous studies have shown that

P. gaeumannii ascospore production (Michaels and Chastagner, 1984), hyphal growth and germination (Capitano, 1999) increase with temperature until maximized at *c.* 20°C. At our sites, during the winter months the observed ambient temperatures were consistently below 20°C, so at the sites with average temperatures nearer the growth optimum (i.e. south coastal sites and north interior site), more rapid colonization rates, as observed, could occur. However, we were unable to determine if the faster rates of *P. gaeumannii* colonization on the warmer plots was due to accelerated rates of hyphal growth or small differences in initial levels of ascospore production and infection rates between north- and south-exposed plots.

The similarity in the patterns of temperature and fungal colonization within plantations suggests a linkage between site temperature and amounts of fungal colonization. Laboratory studies have shown that both temperature and free moisture can influence hyphal growth of *P. gaeumannii* in culture (Capitano, 1999). However, we suspect that typically high amounts of fall-spring rainfall and frequent summer fogs in western Oregon largely negated the importance of differences in free moisture on foliage at these sites. Where moisture is not limiting, temperature is probably the factor having the greatest influence on growth. Furthermore, preliminary field (i.e. nine western Oregon Douglas-fir plantations) and seedling studies also have indicated a direct relationship between site mean daily temperature and *P. gaeumannii* colonization (J. K. Stone and D. K. Manter, unpublished data).

The more severe symptoms frequently observed on south slopes could be attributed to an increase in fungal colonization and/or increased physiological impact. For example, all south slopes had greater needle loss for a given level of pseudothecia abundance compared with their northern counterparts, and at the coastal sites fungal colonization was significantly higher for the south slopes. The exact cause of greater levels of needle loss on south slopes was not studied here, however, it is consistent with the known impacts of *P. gaeumannii* on needle physiology. Due to the reduced photosynthetic rates, and an inability to dissipate excess light energy at high light levels, *P. gaeumannii*-infected needles are susceptible to photo-oxidative damage (Manter, 2002). Therefore, greater amounts of photo-oxidative damage in the south-aspect plots are a possible explanation for the higher levels of needle abscission.

A difference between the ergosterol–pseudothecia density relationship, but not for the quantitative PCR–pseudothecia density relationship, was observed for trees growing on north- and south-aspect plots. Due to the non-species specific nature of the ergosterol technique (i.e. measures all internal and external higher fungi), the plot-specific difference in the ergosterol–pseudothecia density relationship could be due to the presence of other foliar fungi and/or differential pseudothecia development for a given level of *P. gaeumannii* biomass. Manter et al. (2001) suggested that site-to-site variation in the ergosterol–pseudothecia density relationship might be due to the latter, based on the assumption that non-target internal fungal species have a minor contribution to total ergosterol content where *P. gaeumannii* infection is high. However, this interpretation is not consistent with the current study, since there was no difference in the quantitative PCR (i.e. *P. gaeumannii* -specific) – pseudothecia density relationship between plots. Furthermore, Manter et al. (2001) did not quantify the presence of external phylloplane fungi, such as *Rasutoria pseudotsugae* and *Stomiopeltis* sp., which are common inhabitants on the surface of Douglas-fir foliage. Therefore, we suggest that it is the combined presence of internal and external non-target fungal species that results in variation in the ergosterol–pseudothecia density relationship.

The strong correlation between fungal colonization measures indicates that any of these measures may be successfully used in future assays of SNC for both research and management activities. However, we recommend that future studies may benefit from quantifying SNC through pseudothecia density counts for the following reasons: (i) pseudothecia counts had the best correlation with symptom severity, and (ii) a mechanistic pathway relating pseudothecia density and physiological impact exists (Manter et al., 2000). However, under some conditions quantitative PCR may be the most suitable measure of fungal colonization. The benefits of quantitative PCR are: (i) its ability to detect *P. gaeumannii* colonization prior to pseudothecia formation, (ii) it is well correlated with pseudothecia, when present, and (iii) it is relatively inexpensive and non-labour intensive.

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