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The relationship between Swiss needle cast symptom severity and level of *Phaeocryptopus gaeumannii* colonization in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*)*

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Summary

This study examined 108 15-year-old Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) trees to investigate whether trees exhibiting less severe Swiss needle cast (SNC) symptoms were more resistant (had less fungal colonization) or more tolerant (maintained healthy foliage under similar infection levels). Trees were sampled from six open pollinated families that were categorized into three disease severity groups (two families for each group; mild, moderate and severe disease symptoms). The amount of retained foliage and level of discoloration were visually assessed on trees in the field. Fungal colonization (as determined by proportion of stomata occluded with pseudothecia and by amount of *Phaeocryptopus gaeumannii* DNA in sampled needles) was measured on 1- and 2-year-old needles in the laboratory. Trees in the different disease severity groups were similar with respect to amount of fungus in their needles, yet the trees in the mild symptom group retained higher proportions of needles and maintained greener foliage. The relationship between amount of *P. gaeumannii* in needles and SNC symptom severity was statistically significant ($p < 0.05$) for amount of fungal DNA in 1-year-old needles and average needle retention (NR) over the last four growing seasons. Average NR decreased with increased amount of pathogen DNA in the mild disease symptom families. This relationship was reversed in the severe disease symptom group and there was no relationship in the moderate disease symptom group. Because the amount of *P. gaeumannii* DNA in foliage did not differ significantly among the groups, differences in symptom severity were attributed to tolerance, not resistance. Visual scoring of individual trees for average NR over the past four growing seasons could be used to effectively assess for SNC tolerance in Douglas-fir.

1 Introduction

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) that occurs throughout the species' natural range, as well as in areas where Douglas-fir has been cultivated as an exotic species (Morton and Patton 1970; Merrill and Longenecker 1973; Hood and Kershaw 1975; Temel et al. 2003). Although the disease, and the fungus causing it [*Phaeocryptopus gaeumannii* (Rohde) Petrak], have been known to foresters in the Pacific Northwest region of the United States since the early twentieth century (Boyce 1940), SNC was not considered damaging until recently (Hansen et al. 2000). The disease can reduce volume growth by 52% in some coastal Oregon stands (Maguire et al. 2002).

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The major symptoms of SNC include chlorotic foliage and premature abscission of infected needles resulting in sparse tree crowns (HANSEN et al. 2000). Symptom development is closely associated with the obstruction of stomatal function (i.e. gas exchange and water vapour loss control) by pseudothecia, the fruiting bodies of *P. gaeumannii* (MANTER et al. 2000). Airborne spores of the fungus infect newly emerging Douglas-fir needles in the spring and colonize intercellular spaces of needles throughout the summer (CAPITANO 1999). Pseudothecia begin to emerge from stomatal openings between October and February and gradually mature until ascospores are released in May and June. Only newly emerged needles are susceptible to infection by *P. gaeumannii* spores (HANSEN et al. 2000), but hyphae, originating from pseudothecia, continue to grow on needle surfaces and infect additional stomata as needles age (CAPITANO 1999).

No Douglas-fir trees are immune to this pathogen. Nevertheless, there are significant differences in the severity of disease symptoms and growth, even between trees that occupy the same environments. Part of this variation is attributed to genetic variation in Douglas-fir (JOHNSON 2002; TEMEL 2002). The mechanism involved with this variation is unknown. Is it resistance, i.e. do 'healthier' looking trees actually inhibit the level of infection, or is it tolerance, i.e. are 'healthier' looking trees somehow able to continue to grow in the presence of the disease?

A disease symptom is a visible or otherwise measurable adverse change in a plant, produced in reaction to infection by a microorganism or to an unfavourable environmental factor (AGRIOS 2000). A disease sign, however, is the actual presence of the organism causing the disease in the host organism. If a sign can be measured and quantified, it gives an idea on level of infection and colonization of the host by a pathogen. Several methods have been used to assess the amount of colonization by the pathogen and symptom severity in SNC. Methods of assessing colonization include proportion of stomata occluded with pseudothecia (PSOP) (HANSEN et al. 2000) and amount of fungal biomass in the needles determined by quantitative polymerase chain reaction (PCR; WINTON 2001) or ergosterol (MANTER et al. 2001) methods. Quantifiable symptoms of SNC are degree of yellowing of needles and extent of defoliation (needle retention, NR). Several visually scored variables have been employed to assess severity of the SNC symptoms, including: foliage colour (FC), NR and foliage density (FD) (JOHNSON 2002; MAGUIRE et al. 2002). FC is degree of chlorosis in a tree's foliage, NR is proportion of needles retained in a given internode and FD measures density of foliage in the entire crown.

This study was undertaken to better understand the underlying mechanisms affecting variation in SNC symptom severity among different trees, and to compare the relationship between level of *P. gaeumannii* infection and severity of SNC symptoms.

2 Materials and methods

2.1 Plant material

Six open-pollinated Douglas-fir families were selected from two sets of 40 families that had been characterized as having mild, moderate or severe SNC symptoms. Two families were chosen from each disease severity group. Disease severity rating for each family was based on data from two previous SNC and tree growth field assessments conducted in 1995 and 1998 at the Acey Creek (45°45′N, 123°47′W, elevation = 204 m) and Coal Creek (45°46′N, 123°51′W, elevation = 67 m) progeny test plantations, near Tillamook, Oregon, USA, where the effects of the disease are pronounced. Families with mild symptoms were those that had superior diameters and above-average foliage health scores (NR, FC and FD, see Table 1 for definitions and assessment methods). Families with moderate symptoms had average diameters and foliage health scores. Families characterized as having severe

Table 1. **Summary of Swiss needle cast (SNC) symptom and sign variables, and their assessment methods**

Symptoms	Assessment method
Foliage colour (FC)[1]	Visual colour score of overall tree crown from 1 (yellow) to 3 (dark green)
Foliage density (FD)[1]	Visual score of foliage density over entire tree crown from 1 (sparse) to 6 (dense)
Needle colour (NC)	Visually scored from 1 (yellow) to 3 (dark green) on the sampled branch for each of the past four growing seasons (i.e. for 1996, 1997, 1998 and 1999)
Needle retention (NR)[1]	Visually scored from 0 (0–10% retention) to 9 (91–100% retention) on the sampled branch for each of the past four growing seasons (i.e. for 1996, 1997, 1998 and 1999)
Signs	
Percentage stomata occluded with pseudothecia (PSOP) (%)[2]	Proportion of stomata occluded with pseudothecia on the 10 needles, based on 100 stomata per needle
Fungal DNA (DNA) (pg)[2]	Amount of fungal DNA obtained by quantitative PCR

PCR, polymerase chain reaction.
[1]Variables were also assessed in 1995 and 1998 assessments and included in the determination of families for each disease severity group. NR was limited to only first- and second-year needles in 1995 and 1998 assessments.
[2]Variables were assessed for 1999 (PSOP99 and DNA99) and 1998 (PSOP98 and DNA98) needle cohorts separately.

symptoms had poor FC, poor NR and poor growth. This diverse set of families included a range of individual trees from extremely well performing (i.e. good growth, good NR with dense green foliage) to extremely poor performing (i.e. inferior growth, poor NR, with sparse discoloured foliage).

Assessments for this study were conducted on a total of 108 trees from the selected families in Acey Creek and Coal Creek progeny test plantations. Environmental conditions were more or less homogenous within each test site; thus, environmental factors affecting each tree were not expected to vary greatly. The test plantations were established by the Oregon Department of Forestry in 1986 using 1-0 container grown seedlings. Tree spacing was 3 m by 3 m and the sites were fenced to prevent damage by wildlife.

The statistical design of the progeny tests at each site was a reps-in-sets design, such that each set of 40 families was planted together in three replications. Within each replication, a family was represented by a four-tree non-contiguous plot. Because of missing trees in some families in some replications, only three randomly selected trees of the four trees in each replication were assessed. All families had at least three trees in each replication. Each set had one family from each of the disease severity groups; therefore, each severity group was represented, at each site, in six different field replications by three trees.

2.2 Traits and assessment methods

Trees belonging to the six families were visually assessed for SNC symptoms and growth traits in each test plantation, and foliage samples were collected for further analyses in the laboratory. Assessments in the field and sample collection were conducted in March 2000, before budburst (15-year-old trees from seed). FC and FD were assessed on the whole tree, and needle colour (NC) and NR were scored on internodes of the lateral branches representing foliage produced in each of the past four growing seasons (i.e. 1996–99)

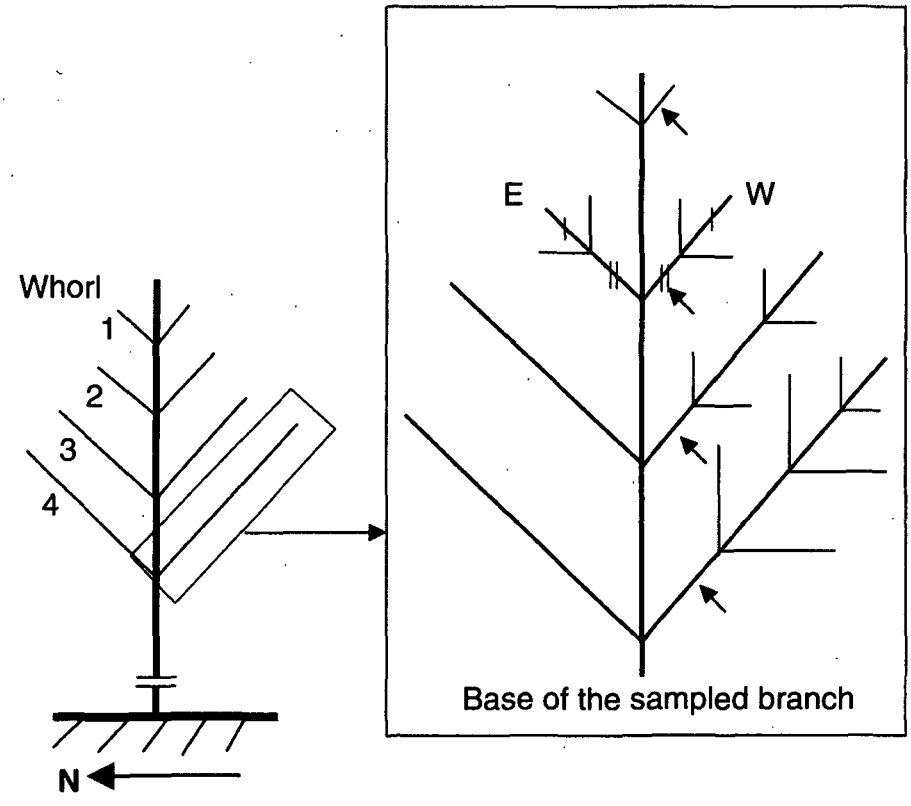

Fig. 1. Illustration of the sampling procedure. Sampled internodes for proportion of stomata occluded with pseudothecia (PSOP) and amount of *Phaeocryptopus gaeumannii* DNA (DNA) assessments are marked (| = 1999 cohort, || = 1998 cohort, E = east, W = west). Arrows indicate internodes scored for needle retention (NR) and needle colour (NC). Also see Table 1

(Table 1, Fig. 1). A south-facing branch in the fourth whorl from the top of each tree was removed for assessment of fungal colonization. South exposed foliage was sampled because disease symptoms are more pronounced on the south side of the upper crown (HANSEN et al. 2000; MANTER et al. 2003a). Care was taken in determining the whorl that was produced as a result of initial bud flush at the beginning of the growing season and not as a result of second flushing.

Two lateral internodes from both 1998 and 1999 growing seasons were clipped from the sampled branch and placed in small plastic bags bearing the location of the internode on the branch ('E' for east and 'W' for west). Samples were kept in an ice chest up to 4 days in the field and transported to Corvallis, Oregon, where they were kept frozen (−10°C) until the laboratory assessments were conducted.

Ten needles (five from each of the east and west internodes) from each of the 1998 and 1999 foliage cohorts (2- and 1-year-old, respectively) were sampled to assess PSOP. The 10 needles were placed side-by-side on a clear glass plate (lower side of needles facing up) with petioles in the same direction. These needles were fixed in their positions by putting another glass plate on them and holding the two plates together with clips. Midpoints of each of the 10 needles between the plates were marked with a pen. Then, the plates were

placed under a dissecting microscope with needle petioles towards the observer. On the second stomata row at the right hand side of the needle midrib, the central 100 stomata were examined for presence or absence of fungal pseudothecia, to estimate the PSOP (HANSEN et al. 2000).

At the same time foliage was sampled for the PSOP assessment, a second set of needles was collected to assess the amount of *P. gaeumannii* DNA (DNA), separately, for the 1998 and 1999 foliage cohorts. Ten randomly selected needles were sampled, five from each cohort, and placed in a screw cap microcentrifuge tube (Island Scientific, Bainbridge Island, WA, USA IS-522) along with two solid glass beads (Fisher Scientific, Hampton, NH, USA 11-312C) for DNA extractions. The tubes were kept frozen at −10°C until DNA extraction.

Total genomic DNA was extracted from foliage and *P. gaeumannii* DNA was specifically amplified and quantified following WINTON et al. (2002). Real-time PCR is a quantitative diagnostic method that can be very useful at very low levels of infection. This technique utilizes Taqman (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) chemistry (LIVAK et al. 1995; GIBSON et al. 1996; HEID et al. 1996) in conjunction with the 7700 Sequence Detection System (PE Applied Biosystems). The fluorogenic Taqman probe, labelled on opposite ends with a reporter dye and a quencher dye, anneals between the PCR primers. During the extension phase of the PCR, the 5′ → 3′ exonuclease activity of *Taq* DNA polymerase cleaves annealed probe molecules. Release of the reporter dye results in a fluorescent signal which is measured by the 7700 Sequence Detection System during each cycle of the PCR process.

Each sample was subjected to PCR twice. Because of large sample size PCR was conducted in several batches. In order to remove batch effects, results for each sample were multiplied by the ratio between mean of four standards in that batch and mean of all mean standards in all batches. Then average of the two results was used in the analyses.

2.3 Data analysis

Needle retention and NC were averaged over the last four growing seasons and these averages were used throughout the analyses. PSOP and DNA were assessed on mean values of the 10-needle samples for 1998 and 1999 foliage cohorts. Preliminary investigations indicated that DNA and PSOP violated the normality and constant variance assumptions of ANOVA and were transformed to natural logarithms before analyses (STEEL and TORRIE 1980). All reported mean values, however, are before transformation, unless otherwise noted. Differences were deemed significant at $p = 0.05$. Analyses were conducted to test differences among the disease severity groups, not among families. The error variances were consistent from site to site for each variable.

2.3.1 Objective 1: Determining resistance/tolerance mechanism

In order to investigate amount of variation and differences among the disease severity groups, variable mean values and ranges were calculated and ANOVAS were carried out for all variables using the generalized linear models (GLM) procedure of the SAS statistical package (SAS INSTITUTE INC. 1990) according to following mixed model:

$$y_{ijkl} = \mu + s_i + r_{j(i)} + \tau_k + \mathrm{sd}_{ik} + \mathrm{rd}(s)_{jk(i)} + e_{ijkl} \quad (1)$$

where, y_{ijkl} is the value of the lth tree of the kth disease severity group in the jth replication of the ith site; μ is the overall mean; s_i is the effect of the ith site, $E(s_i) = 0$, $\mathrm{Var}(s_i) = \sigma_s^2$; $r_{j(i)}$ is the effect of the jth replication at the ith site, $E(r_{j(i)}) = 0$, $\mathrm{Var}(r_{j(i)}) = \sigma_r^2$; τ_k is the fixed effect of the kth disease severity group, $\Sigma_{k=1}^{3}\tau_k = 0$; sd_{ik} is the interaction between the ith site and the kth disease severity group, $E(\mathrm{sd}_{ik}) = 0$, $\mathrm{Var}(\mathrm{sd}_{ik}) = \sigma_{\mathrm{sd}}^2$; $\mathrm{rd}(s)_{jk(i)}$ is the

interaction between the kth disease severity group and the jth replication in the ith site, $E(rd(s)_{jk(i)}) = 0$, $Var(rd(s)_{jk(i)}) = \sigma^2_{rd(s)}$; and e_{ijkl} is the random error, $E(e_{ijkl}) = 0$, $Var(e_{ijkl}) = \sigma^2_e$.

Another linear model was used for the data collected at each site to determine the differences between the 1999 and 1998 foliage for each variable assessed in the laboratory.

$$y_{jklm} = \mu + r_j + \tau_k + \alpha_l + \tau\alpha_{kl} + e_{jklm} \quad (2)$$

where, y_{jklm} is the value of the lth age in the mth tree of the kth disease severity group in the jth replication; μ is the overall mean; r_j is the effect of the jth replication, $E(r_j) = 0$, $Var(r_j) = \sigma^2_r$; τ_k is the fixed effect of the kth disease severity group, $\Sigma^3_{k=1}\tau_k = 0$; α_l is the fixed effect of the lth age, $\Sigma^2_{l=1}\alpha_l = 0$; $\tau\alpha_{kl}$ is the interaction effect between kth disease severity group and lth age, $\Sigma^3_{k=1}\Sigma^2_{l=1}\tau\alpha_{kl} = 0$; and e_{jklm} is the random error, $E(e_{jklm}) = 0$, $Var(e_{jklm}) = \sigma^2_e$.

2.3.2 *Objective 2: Investigating the relationship between level of infection and symptom severity*

Regression was used to investigate the relationship between symptoms (NR, FC, NC and FD) and level of infection (DNA or PSOP). The full regression model was:

$$\text{Symptom} = \text{SITE}_i + \text{REPLICATION}_{ij} + \beta_1(\text{Infection}) + \text{GROUP}_k + \beta_k(\text{GROUP}) \quad (3)$$

where, SITE_i is the effect of the ith site, REPLICATION_{ij} is the effect of the jth replication on the ith site, β_1 indicates the overall relationship (slope) between the symptom in question and the particular measurement of infection. The GROUP_k effect indicates whether there is a difference in the relationship, the intercept in this case, for the three severity groups. The β_k (GROUP) term indicates whether the slope of the relationship differs for the different severity groups. The final models only included effects that were significant at the $p = 0.05$ level.

3 Results

3.1 Objective 1

Overall, severity of the disease symptoms was similar at both sites. Symptoms were slightly more severe at Acey Creek, which had lower average NR than Coal Creek (Table 2). Pseudothecia and fungal DNA were present in all sampled needles, indicating that all trees included in this study were infected by *P. gaeumannii*. Both PSOP and DNA were highest in the severe symptom group (except for PSOP in 2-year-old needles) and the lowest in the mild symptom group (Table 3), but differences among groups were not statistically significant for these traits.

As expected, the severity of disease symptoms increased from the mild symptom group to the severe symptom group. Differences among groups were significant for NR, FC and FD. However, it should be noted that considerable variation was observed within each group for all three of these symptoms. Colonization of foliage by *P. gaeumannii*, represented by DNA and PSOP, was greater in 2-year-old (1998) needles than in 1-year-old (1999) needles in all disease severity groups (Table 3).

3.2 Objective 2

A significant relationship was found between infection level and severity of disease symptoms for NR and log (DNA99) (amount of *P. gaeumannii* DNA in needles

Table 2. **Site mean values (and SD) of SNC symptom and sign variables included in this study**

Symptoms	Acey Creek	Coal Creek
FC	2.39 (0.74)	2.35 (0.65)
FD	3.67 (1.26)	3.31 (1.36)
NC	1.50 (0.47)	1.51 (0.45)
NR*	4.05 (1.33)	4.43 (1.48)
Signs		
PSOP99**	3.69 (2.75)	2.56 (1.81)
PSOP98	10.71 (4.79)	8.42 (3.76)
DNA99**	111.05 (140.72)	90.22 (95.02)
DNA98	302.06 (231.77)	264.24 (150.95)

SNC, Swiss needle cast; FC, foliage colour; FD, foliage density; NC, needle colour; NR, needle retention; PSOP, percentage stomata occluded with pseudothecia.
*Significant site differences ($p = 0.05$).
**Significant differences between 1999 and 1998 needle cohorts ($p = 0.05$) for PSOP and DNA at each test site.

Table 3. **Mean values (and SD) of SNC symptom severity and sign variables included in this study for each disease severity group**

	Disease severity group				
Symptoms	Mild	Moderate	Severe	*F*-value	Probability > *F*-value
FC	2.78 (0.42)	2.30 (0.71)	2.03 (0.70)	79.86	0.0124
FD	4.39 (0.96)	3.17 (1.11)	2.92 (1.36)	185.62	0.0054
NC	1.63 (0.44)	1.51 (0.43)	1.38 (0.49)	8.45	0.1058
NR	4.88 (1.21)	4.05 (1.44)	3.81 (1.40)	528.41	0.0019
Signs					
PSOP99	2.63 (1.93)	3.18 (2.17)	3.57 (2.90)	0.79	0.5589
PSOP98	9.18 (3.77)	9.88 (4.30)	9.65 (5.22)	1.58	0.3877
DNA99	91.33 (125.92)	100.74 (93.74)	109.84 (138.46)	1.36	0.4234
DNA98	257.77 (160.59)	289.76 (210.19)	301.92 (214.27)	0.26	0.7915

Mild, green foliage and good needle retention with good tree growth; moderate, yellowish green foliage and moderate needle retention with moderate tree growth; severe, yellow foliage and poor needle retention with poor tree growth.
SNC, Swiss needle cast; FC, foliage colour; FD, foliage density; NC, needle colour; NR, needle retention; PSOP, percentage stomata occluded with pseudothecia.

produced in 1999) (Fig. 2). For all other disease symptoms and disease signs, significant terms in regression models included either the intercept alone or the intercept with group, where severity of the symptoms increased from the mild to severe symptom group. Average NR decreased with increased DNA99 in the best performing (i.e. mild symptom group) families. This relationship was reversed in the severe symptom families. In the moderate symptom families, NR was not correlated with the amount of fungal colonization (Fig. 2).

4 Discussion

Of the 108 individuals tested, all were infected with *P. gaeumannii* at similar levels. There was no evidence of quantitative or absolute (immune) resistance. However, Douglas-fir genotypes appear to vary in their ability to tolerate colonization by *P. gaeumannii*. The

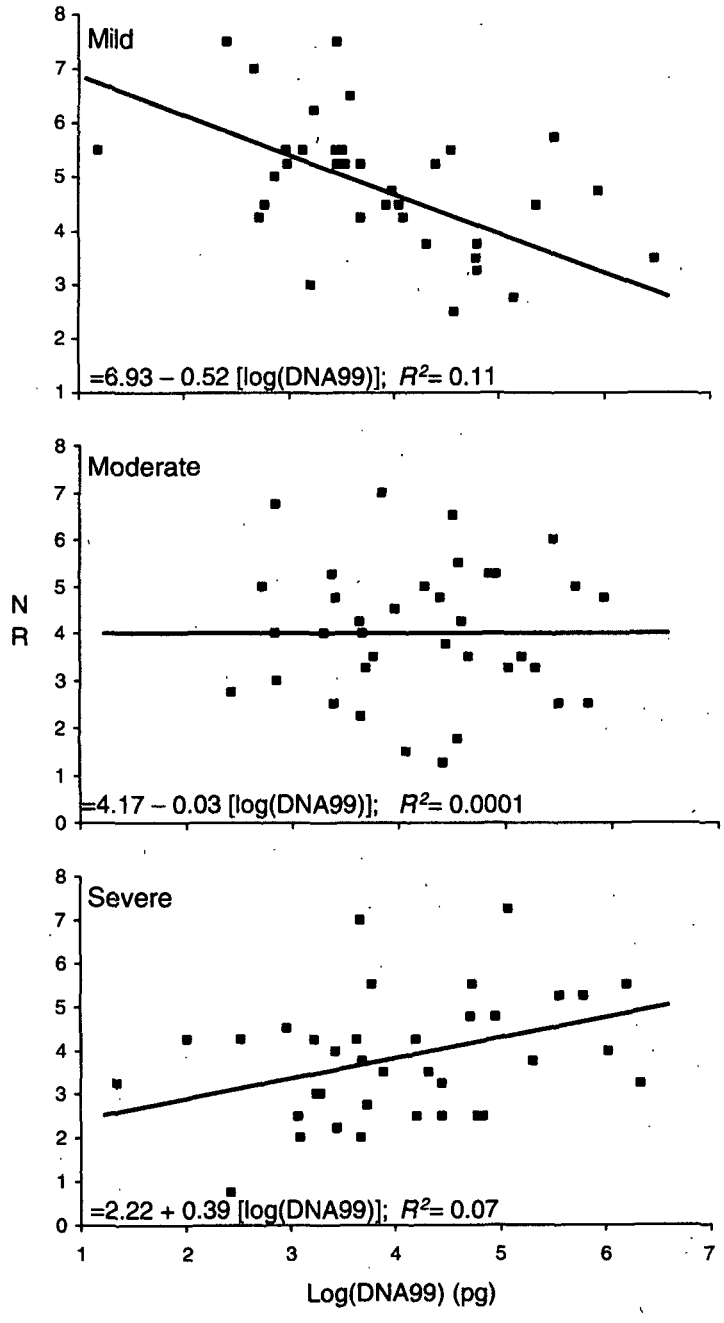

Fig. 2. Relationship between natural logarithm of *Phaeocryptopus gaeumannii* DNA content in 1999 needles [log(DNA99)] and average needle retention of the last four growing seasons (NR) for mild, moderate and severe disease severity groups. Regression equations and their R-squares are given for each disease severity group

pathogen seems to infect the needles and grow in infected needles at a similar pace even in the seemingly most tolerant Douglas-fir families. This is in agreement with a study that investigated 55 seedling families of Douglas-fir and found no family differences with respect to infection (TEMEL 2002). Genetic variation in SNC symptom severity among individual Douglas-fir trees therefore appears to be due to variation in tolerance, not resistance.

Initial infection of foliage by *P. gaeumannii* occurs early in the first growing season after buds open. Colonization of the needles occurs by growth of fungal hyphae in intercellular spaces and increases throughout the lifetime of the needle until it is shed (CAPITANO 1999). The greater amount of *P. gaeumannii* colonization observed in 1998 *vs.* 1999 needles is in agreement with the results obtained in previous studies (CAPITANO 1999; MAGUIRE et al. 2002), indicating that fungal colonization increases continuously during the lifetime of the needle.

The variation in SNC tolerance in Douglas-fir appears to have developed as a result of variation in environmental conditions at Douglas-fir growing locations. A general pattern has been noted where susceptibility to infection by *P. gaeumannii* and symptom severity tend to be greater in Douglas-fir genotypes originating from areas of lower rainfall and humidity (HOOD 1982; MCDERMOTT and ROBINSON 1989). HOOD (1982) reported a significant regional correlation between mean spring rainfall and incidence of infection. This pattern led MCDERMOTT and ROBINSON (1989) to suggest that higher disease pressure in geographic locations of high rainfall and humidity has led to greater selection pressure for disease resistance to SNC in local natural populations. This hypothesis is supported by a later study conducted in a provenance trial planted in Germany and representing Douglas-fir provenances from throughout North America. In this study, NR was higher for provenances of the coastal (or green) variety (var. *menziesii*), which naturally grows in areas of generally higher rainfall than for provenances of interior grey and blue varieties (var. *caesia* and var. *glauca*, respectively) in the presence of SNC (STEPHAN 1997).

Several hypotheses have been proposed for factors influencing leaf lifespans in plants (CHABOT and HICKS 1982). Many of these hypotheses are based on cost and benefit relationship in producing and maintaining foliage (JOHNSON and TIESZEN 1976; MILLER and MOONEY 1976). In general, when the initial cost of foliage production is high and photosynthetic rate is low the foliage is retained longer. It has been shown that the photosynthetic rate of *P. gaeumannii*-infected Douglas-fir needles decreases with increasing *P. gaeumannii* colonization (MANTER et al. 2000), reducing leaf productivity per unit of time. The mechanism(s) involved in SNC tolerance seem to be associated with reduced photosynthetic rates of colonized needles. The trees with mild symptoms, i.e. most tolerant, are least susceptible to losing their needles overall, i.e. have highest mean NR, but are more susceptible to needle loss with increased levels of infection. This way, a needle is shed when cost of maintaining it exceeds the benefits from retaining it. In fact, MANTER et al. (2003b) reported that abscission of *P. gaeumannii*-infected older Douglas-fir needles reduces the losses in net CO_2 assimilation by 31.8%. It is also possible that photosynthetic rates of needles of the tolerant families are higher than that of needles in the less-tolerant families, so between the time they are produced and shed that they can return the investment made by the tree to produce them and contribute to tree growth.

Since NR was the only trait that appeared to be related to level of infection, this trait may be the best indicator of tolerance to SNC in field and can be employed to assess SNC severity in future studies. MAGUIRE et al. (2002) also found that foliage loss (NR) was a reliable indicator of growth reduction. Needle retention is probably the one trait examined that represented various tolerance mechanisms. Assessing NR over the last 4 years may provide a more robust indicator of tolerance than only assessing 1 or 2 years retention

because NR was higher in all needle cohorts in the mild symptom group than in the other two groups. Needle cohorts older than 4 years often are absent in infected trees (MAGUIRE et al. 2002) and so cannot be used for comparisons.

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Résumé

Relation entre la sévérité des symptômes de rouille suisse sur aiguilles et le niveau de colonisation par Phaeocryptopus gaeumannii chez le Douglas (Pseudotsuga menziesii var. menziesii)

L'étude a porté sur 108 Douglas (*Pseudotsuga menziesii* var. *menziesii*) âgés de 15 ans pour déterminer si les arbres montrant les symptômes les moins sévères de rouille suisse étaient les plus résistants (avec une moindre colonisation fongique) ou les plus tolérants (maintenant une qualité de feuillage supérieure à niveau d'infection équivalent). Les arbres ont été échantillonnés dans 6 familles issues de pollinisation libre classées dans 3 groupes de sévérité de la maladie (2 familles dans chaque groupe; symptômes légers, modérés ou sévères). La quantité d'aiguilles restantes et les altérations de couleur ont été estimées visuellement sur le terrain. La colonisation fongique (définie comme la proportion de stomates obstrués par des pseudothécies et par la quantité d'ADN de *Phaeocryptopus gaeumannii* dans les aiguilles échantillonnées) a été mesurée au laboratoire sur des aiguilles d'un an et de deux ans. Les arbres appartenant à différents groupes de sévérité de maladie n'ont pas présenté de différence pour la quantité de champignon dans les aiguilles. Cependant, les arbres du groupe à symptômes légers ont conservé une plus grande proportion d'aiguilles et un feuillage plus vert. La relation entre la quantité de *P. gaeumannii* dans les aiguilles et la sévérité des symptômes est significative ($p < 0.05$) lorsque l'on considère la quantité d'ADN fongique dans les aiguilles d'un an et la rétention moyenne des aiguilles au cours des quatre saisons de croissance précédentes. La rétention moyenne des aiguilles décroît avec la quantité d'ADN fongique chez les familles à symptômes légers. La relation inverse est observée dans le groupe à symptômes sévères, et aucune relation dans le groupe à symptômes modérés. Puisque la quantité d'ADN de *P. gaeumannii* dans les aiguilles ne diffère pas significativement entre groupes, les différences de sévérité des symptômes sont attribuées à des différences de tolérance et pas de résistance. La notation visuelle de la rétention des aiguilles par arbre au cours des quatre dernières saisons pourrait être utilisée pour estimer la tolérance du Douglas à la rouille suisse.

Zusammenfassung

Zusammenhang zwischen der Symptomausprägung der Russigen Nadelschütte und dem Ausmass der Besiedelung durch Phaeocryptopus gaeumannii bei Pseudotsuga menziesii var. menziesii

An 15jährigen Douglasien (*Pseudotsuga menziesii* var. *menziesii*, n = 108) wurde geprüft, ob Bäume mit geringerer Symptomausprägung der Russigen Nadelschütte (Swiss needle cast, SNC) resistenter (d.h. geringere Pilzbesiedelung aufweisen) oder toleranter sind (d.h. die Nadeln bei ähnlicher Besiedelung durch den Pilz symtomfrei bleiben). Es wurden Bäume aus sechs offen bestäubten Familien beprobt, die in drei Symptomklassen gruppiert wurden (zwei Familien in jeder Gruppe; schwache, mittlere und starke Symptome). Die Benadelung und die Nadelverfärbung wurden im Feld visuell bonitiert. Die Pilzbesiedelung (Anteil mit Pseudothecien verschlossener Spaltöffnungen und Menge von *Phaeocryptopus gaeumannii* – DNA in den Nadeln) wurde an ein- und zweijährigen Nadeln im Labor bestimmt. Die Bäume der verschiedenen Symptomklassen hatten ähnlich viele Fruchtkörper auf ihren Nadeln, auch wenn die Bäume der schwächsten Symptomklasse die Nadeln länger behielten und grünere Belaubung zeigten. Die Beziehung zwischen dem Vorkommen von *P. gaeumannii* in den Nadeln und der Symptomausprägung, gemessen am Pilz-DNA-Gehalt in den einjährigen Nadeln und der durchschnittlichen Nadelretention über die letzten 4 Jahre, war statistisch signifikant ($p < 0.05$). In Familien der schwachen Symptomklasse nahm die durchschnittliche Nadelretention mit zunehmendem Gehalt an Pilz-DNA ab. Diese Beziehung war in der stärksten Symptomklasse gerade umgekehrt, in der mittleren Klasse war keine Beziehung nachweisbar. Da sich in den unterschiedlichen Symptomklassen die Gehalte der *P. gaeumannii*-DNA in den Nadeln nicht signifikant unterschieden, werden die Symptomunterschiede als Toleranz, nicht Resistenz interpretiert. Eine visuelle Bonitierung der einzelnen Bäume bezüglich Nadelretention während der letzten vier Wachstumsperioden ermöglicht eine effiziente Bewertung der SNC-Toleranz von Douglasien.

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