Early genetic testing of coastal Douglas-fir for Swiss needle cast tolerance[1]

Fatih Temel, G.R. Johnson, and W.T. Adams

Abstract: The possibility of early testing coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) for Swiss needle cast (SNC; caused by *Phaeocryptopus gaeumannii* (Rohde) Petrak) tolerance was investigated using 55 Douglas-fir families from western Oregon. Seedlings were inoculated with *P. gaeumannii* naturally in the field and were visually scored for a variety of SNC symptom traits (i.e., needle and foliage color, and retention) at the seedling stage (age 2) and in "mature" (ages 10 and 12) trees at two test sites for both the seedling and mature ages. Seedlings were also assessed in the laboratory for SNC symptom traits, for proportion of needle stomata occluded with pseudothecia (PSOP), and for amount of *P. gaeumannii* DNA in needles. Although families differed significantly at both ages for all SNC symptom traits and for PSOP, they did not differ for amount of fungal DNA. Thus, genetic variation in SNC symptoms appears to be primarily due to differences in tolerance to the disease rather than to resistance to infection per se. Estimated individual-tree heritabilities for SNC symptom traits were low to moderate (mean h_i^2 = 0.19, range 0.06–0.37) at both ages, and within each age-class these traits were moderately to strongly genetically correlated (mean r_A = 0.69, range 0.42–0.95). Type B genetic correlations between SNC symptom traits in seedlings and mature trees ranged from 0 to 0.83 and were weakest for traits measured in the laboratory. Genetic gain estimates indicated that family selection for SNC tolerance (i.e., greener needles or greater foliage retention) at the seedling stage can be very effective in increasing tolerance in older trees.

Résumé : La possibilité d'utiliser un test précoce pour évaluer la tolérance du douglas de Menzies typique (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) au rouge de Gaeumann (*Phaeocryptopus gaeumannii* (Rohde) Petrak) a été évaluée à l'aide de 55 familles de douglas. Des semis ont été inoculés naturellement avec *P. gaeumannii* au champ et ont été visuellement évalués pour une variété de symptômes du rouge (couleur des aiguilles et rétention du feuillage) chez des semis (2 ans) et des arbres plus vieux (10 et 12 ans). Les semis ont été évalués en laboratoire pour les symptômes de la maladie, la proportion de stomates obstrués par des pseudothèces et la quantité d'ADN de *P. gaeumannii* dans les aiguilles. Il y a des différences significatives entre les familles aux deux âges pour tous les symptômes de la maladie et la proportion de stomates obstrués par des pseudothèces mais non pour la quantité d'ADN fongique. Par conséquent, les variations génétiques dans les symptômes de la maladie semblent dues à la tolérance plutôt qu'à la résistance proprement dite. Chez les arbres pris individuellement, l'héritabilité estimée pour les symptômes de la maladie était faible à modérée (h_i^2 moyen = 0,19; écart de 0,06 à 0,37) aux deux âges, et la corrélation génétique entre ces symptômes était modérée à forte à l'intérieur de chaque classe d'âge (r_A moyen = 0,69; écart de 0,42 à 0,95). Les corrélations génétiques de type B entre les symptômes de la maladie chez les semis et les arbres plus vieux variaient de 0 à 0,83. Les estimations de gain génétique indiquent que la sélection de familles pour la tolérance à la maladie (c.-à-d., des aiguilles plus vertes ou une meilleure rétention du feuillage) à l'état de semis peut être très efficace pour augmenter la tolérance chez les arbres plus vieux.

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Introduction

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) caused by an ascomycete, *Phaeocryptopus gaeumannii* (Rohde) Petrak. Airborne spores of the fungus land and germinate on newly emerging Douglas-fir needles in the spring, and hyphae grow into the stomata. Infection and colonization of the needles by the fungus continue throughout summer. In coastal Oregon, fungal fruiting bodies (i.e., pseudothecia) can emerge from the stomata between October and February, obstructing the ability of needles to control water vapor loss and gas ex-

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F. Temel[2,3] and W.T. Adams. 321 Richardson Hall, Department of Forest Science, Oregon State University, Corvallis, OR 97731, USA.
G.R. Johnson. USDA Forest Service, Pacific Northwest Research Station, 3200 Southwest Jefferson Way, Corvallis, OR 97331, USA.

[1]This is Oregon State University Forest Research Laboratory Research Paper 3611.
[2]Corresponding author (e-mail: fatihtemel93@yahoo.com).
[3]Present address: Kafkas Üniversitesi, Artvin Orman Fakültesi, 08000 Artvin, Turkey.

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change (Manter et al. 2000). Hyphae grow from the fruiting bodies on lower needle surfaces and infect additional stomata (Capitano 1999). Ascospore release from the fruiting bodies starts in March and peaks in midsummer (Michaels and Chastagner 1984; Stone et al. 1999). Infected needles discolor and are abscised prematurely the following summer (Hansen et al. 2000), resulting in significant stem growth loss (Beekhuis 1978; Maguire et al. 2002).

Although the disease is native to the Pacific Northwest region of the United States and has been known to foresters since the early 1900s (Boyce 1940), it was not a major concern. Therefore, Douglas-fir breeding programs in the region have not included tolerance to this disease as a selection criterion, primarily focusing on other economically important traits, such as growth, form, and wood density. In the late 1980s, however, SNC became problematic when it was associated with significant growth loss in Douglas-fir plantations located in coastal Oregon and Washington (Kanaskie et al. 1996; Hansen et al. 2000). Maguire et al. (2002) reported a 23% average growth reduction in diseased Douglas-fir stands in coastal Oregon.

All Douglas-fir trees appear to be susceptible to infection by *P. gaeumannii*, but severity of the disease symptoms (e.g., yellowing and loss of needles) varies from tree to tree (Temel 2002; Temel et al. 2004). No evidence of immunity has been found.

A few reports on genetic variation in SNC severity symptoms are available in the literature. Nelson et al. (1989) found differences in density of stomata occluded with pseudothecia among 20 full-sib families in a Christmas tree plantation in Oregon. Similarly, significant clonal differences were observed in "attack index" among 130 Douglas-fir clones in Romania (Blada 1988). McDermott and Robinson (1989) observed that both needle retention and proportion of needles with fruiting bodies varied among nine Douglas-fir provenances (range from southern California to British Columbia) tested in British Columbia. Similar results were obtained from Douglas-fir provenance tests in New Zealand (Hood and Wilcox 1971) and Germany (Stephan 1997). Johnson (2002) found that needle retention, crown density, and foliage color differed among 505 wind-pollinated Douglas-fir families in seven progeny test plantations in coastal Oregon. Blada (1988) reported very high broad-sense heritability estimates for SNC disease symptoms (>0.80), but Johnson (2002) found symptoms to be under weak genetic control (narrow- sense heritability estimates ranged from 0.14 to 0.25). Therefore, there is a potential for breeding Douglas-fir for SNC tolerance, but the strength of inheritance of SNC traits is not clear.

Early testing for SNC tolerance has a number of potential advantages: (1) early testing could be used to reduce breeding cycle length as long as other important traits can also be assessed in young trees, (2) efficiency of testing can be increased because testing can occur in a smaller area, reducing environmental variation among tested trees, and (3) if trees must be tested over a longer period of time in the field for other traits (e.g., stem growth and form), the size of these tests could be reduced by removing susceptible families prior to outplanting. In addition, the disease symptoms can be assessed easily and probably more reliably in small trees than in larger trees. There could also be additional traits that would predict tolerance in seedlings but are expensive to measure in older trees. Overall, genetic gains can be maximized by incorporating early selection schemes into breeding programs (Lowe and vanBuijtenen 1989).

Several conditions must be met if early testing for SNC tolerance is to be successful. First, the seedlings should be uniformly infected by *P. gaeumannii*. Second, once seedlings are successfully inoculated and disease symptoms develop, the appropriate traits to assess need to be determined. Selected traits should possess several features. They should be heritable, represent actual tolerance, be good predictors of future performance (i.e., good genetic correlation with SNC tolerance in older trees), and selection for these traits should not adversely affect other traits of interest. Appropriate timing of assessment is also important because, while infection takes place primarily in May and June, colonization of needles happens throughout the year and severity of disease symptoms increases with time. In addition, assessment methods should be practical and inexpensive.

Several traits are currently employed to assess severity of SNC based on disease symptoms and signs. Symptoms are the host's reactions to invasion by a pest, whereas signs are the visible and (or) quantifiable existence of the pest organism on the host (Agrios 2000). Yellowness, density of foliage, and proportion of retained needles are symptoms that are frequently assessed in trials (McDermott and Robinson 1989; Nelson et al. 1989; Hansen et al. 2000; Johnson 2002). Proportion of stomata occluded with pseudothecia and amount of fungal biomass quantified either by quantitative polymerase chain reaction (PCR) (Winton et al. 2002) or by ergosterol analysis (Manter et al. 2001) are commonly accepted as signs of SNC. While the sign traits are continuous variables, subjective visual scoring (categorical) assessment methods are employed to assess symptoms. Visual assessments are quick and less labor intensive, but because they are subjective, their value may be questioned.

The goals of this study were (1) to determine suitable SNC tolerance assessment traits for early testing purposes, (2) to evaluate quantitative genetics of these traits, and (3) to examine efficiency of early selection for SNC tolerance in coastal Douglas-fir.

Materials and methods

Fifty-five open-pollinated Douglas-fir families were included in this study. The parent trees were selected from a USDA Forest Service (USFS) breeding program in the Hebo Ranger District of the Siuslaw National Forest in coastal Oregon, USA. Parent tree locations ranged in latitude from 44°55′N to 45°20′N, in distance to the Pacific Ocean from 6.3 to 23.6 km, and in elevation from 162 to 442 m. Individual trees from the 55 families were assessed at two 2-year-old (i.e., juvenile) seedling trials (Pleasant Valley and Toledo).

Trees from the same 55 families were also assessed at ages 10 and 12 in two "mature" progeny tests (Salal and Gordy). In the context of early selection, "mature" would usually refer to rotation age. While 12 years is only about one-fourth the rotation age for aggressively managed Douglas-fir, it has been suggested that selections be completed before age 15 to maximize genetic gain per unit of time (Johnson et

Table 1. Site means for Swiss needle cast traits included in this study for juvenile and mature trials.

	Juvenile trials					
	Field assessments		Laboratory assessments		Mature trials	
	Pleasant Valley	Toledo	Pleasant Valley	Toledo	Gordy	Salal
Needle color[a]	1.77, **1.53**	2.01, **1.58**	2.60	2.75	—	—
Needle retention[a]	7.40, **3.25**	8.34, **4.12**	7.17	7.53	8.32	6.44
Foliage color[b]	1.84, **1.56**	2.12, **1.60**	—	—	2.00	1.97
Foliage density[b]	4.42, **2.25**	4.92, **2.68**	—	—	3.62	3.52
PSOP[c]	—	—	30.72	34.80	—	—
Fungal DNA[d]	—	—	571.10	754.80	—	—

Note: All values are for summer assessments except for those in bold, which are fall assessments in the juvenile trials. "—", not measured.

[a]Visual assessment of needle color and retention on a sample branch (see text).

[b]Visual assessment of foliage color and density over the entire crown (see text).

[c]Proportion of stomata occluded with pseudothecia.

[d]Amount of *Phaeocryptopus gaeumannii* DNA (picograms) in 10 needles.

al. 1997). Therefore, for simplicity, these plantations will be called "mature" test sites.

Juvenile trials

Plant material to establish the juvenile trials was sown in the winter of 1998 and grown in a greenhouse at the USFS Dorena Tree Improvement Center near Cottage Grove, Oregon, following standard nursery practices for Douglas-fir (Temel 2002). After germination, seedlings were randomly assigned to eight replicated blocks where each of the 55 families was represented by a five-tree row plot. Integrity and layout of the blocks were maintained throughout the study. In January 1999, the 1-year-old seedlings were put into cold storage (4 °C) for 4 months. Chilling of seedlings is an effective way of delaying dehardening and thus promoting uniform bud burst when seedlings are outplanted (Campbell and Sugano 1975).

Two seedling trials were established in coastal Oregon in May 1999, one in Pleasant Valley (45°21′N, 123°48′W, elevation 62 m), near Tillamook, and the other in Toledo (44°37′N, 123°57′W, elevation 110 m), near Newport.

Following outplanting of the study trees, competing vegetation was removed by hand in Pleasant Valley (primarily grass) and by herbicide treatment in Toledo (primarily *Rubus* sp.). Remnants of thick competing vegetation within the plot at Toledo blocked air circulation around trees and created ever-present wet conditions, thus predisposing the trees to a common nursery disease, gray mould, caused by *Botrytis cinerea* Pers.: Fr. A total of 631 trees were affected, and 58 trees were killed by gray mould at Toledo (total number of dead trees = 131, or 12%). Seventeen (0.02%) trees were lost at Pleasant Valley.

The statistical design at each site was a randomized complete block comprising four blocks. Spacing was 60 cm (at Pleasant Valley) or 45 cm (at Toledo) between rows and 30 cm between trees within rows. Soil conditions were highly heterogeneous at Toledo, thus spacing between rows was reduced at this site to maintain each replication in a smaller, more homogenous area. Both sites were fenced to prevent animal damage. The seedlings in Pleasant Valley and Toledo test sites were naturally infected with *P. gaeumannii* spores released from surrounding heavily infected Douglas-fir stands.

Mature progeny tests

The mature progeny test plantations were also located in coastal Oregon, in the Siuslaw National Forest (Salal: 45°08′N, 123°53′W, elevation 100 m; Gordy: 44°55′N, 123°57′W, elevation 265 m). These progeny tests were established in 1986 using 1–0 container-grown seedlings. Survival of the trees at the time of the SNC assessments (age 12) was 67% at Salal and 87% at Gordy. Trees in these plantations were naturally infected with *P. gaeumannii*.

The statistical design was "sets-in-replications". At each site, three sets were randomly assigned to three sub-blocks within each of five replicated blocks. The 55 families used in this study were selected from two of the three sets (28 families from one set and 27 families from another set). Families were represented by a single four-tree noncontiguous plot within each sub-block. Tree spacing was 3 m × 3 m at planting. The tests were fenced to prevent animal damage.

Objective 1: determination of suitable SNC traits for early testing purposes

Douglas-fir individuals in the seedling trials and mature progeny tests were assessed for SNC symptom traits in the field (Table 1). Additional assessments were conducted in the laboratory on needle samples collected from the seedlings. Height was measured in the mature trials at age 10, and diameter at breast height (DBH) was measured at age 13.

In the summer of 2000 (between 29 May and 9 June), seedlings (2 years old) at both Pleasant Valley and Toledo were visually scored for needle color (from 1 = yellow to 3 = dark green), needle retention (from 0 = <10% retention to 9 = 91%–100% retention), foliage density (from 1 = sparse to 6 = dense), and foliage color (from 1 = yellow to 3 = dark green). Needle color and needle retention were assessed on 1999 cohort needles on a south-facing branch. Foliage density and foliage color were assessed over the entire crown of the seedlings. Field assessments in the seedling trials were repeated in the fall (September 2000), after bud set.

After the summer field assessments were completed in the seedling trials, needle samples were collected for laboratory analysis. An internode with 1999 cohort needles on a south-facing branch was collected from each seedling, placed in a

plastic bag, kept in an ice-chest in the field, and stored in a freezer at –10 °C until laboratory analyses.

Laboratory samples were assessed for needle color, needle retention, proportion of stomata occluded with pseudothecia (PSOP), and amount of fungal DNA in the needles. Needle color was visually scored on a scale from 1 (yellow) to 4 (dark green) by comparing the sample color with a color photo of needles exhibiting four distinct color categories. In a preliminary sample of 50 internodes, needle retention, determined by dividing the number of needles present by the total number of positions for needles, was compared with visual estimation (described previously for the field assessment). The correlation between the two assessments was near unity (0.97, $P < 0.01$); thus, the less laborious visual assessment was employed in determining needle retention in the laboratory.

From each individual internode sample, two needles were randomly selected per tree (10 needles per family plot) and placed between two 10 cm × 20 cm clear glass plates. The plates were placed under a dissecting microscope, with needle petioles facing the observer. On the third stomata row on the right-hand side of the needle midrib, the central 100 stomata were examined (1000 stomata per family plot) for presence or absence of fungal pseudothecia to estimate PSOP.

To quantify the amount of fungal DNA in Douglas-fir needles, two random needles from each sample internode (10 needles per family plot) were collected and kept in the freezer until extraction. Total genomic DNA extraction and quantification of *P. gaeumannii* DNA using real-time PCR were carried out according to Winton et al. (2002).

The mature progeny tests were visually assessed in the field twice, in the summers of 1996 and 1998 (at ages 10 and 12, respectively). Each tree was scored for foliage density and foliage color based on the entire tree crown using the same field scoring systems described for seedlings. Needle retention was scored on branch internodes produced in the growing season previous to the time of scoring, again using the methods described for seedlings, except that scoring was conducted on a random branch internode in the upper third of each tree crown. Needle color was not scored on individual branch internodes, nor were laboratory assessments conducted for the mature tests.

Objective 2: evaluating quantitative genetics of SNC assessment traits

Data analyses were conducted using the SAS statistical package (SAS Institute Inc. 1990). All traits (Table 1) were subjected to analysis of variance (ANOVA), first for each individual test and then with the data sets combined over field sites in each age group. Since error variances were consistent for each trait from site to site in each group, there was no adjustment necessary to combine the data over sites (Temel 2002).

Because of differences in statistical designs used in the seedling and mature progeny tests, different random models were employed. The linear model for the combined data over the juvenile trials was

$$[1] \quad y_{ijkl} = \mu + s_i + r(s)_{j(i)} + f_k + sf_{ik} + rf(s)_{jk(i)} + e_{ijkl}$$

where y_{ijkl} is the observation on the lth tree of the kth family in the jth replication in the ith site; μ is the overall mean; s_i is the effect of the ith site; $r(s)_{j(i)}$ is the effect of the jth replication at the ith site; f_k is the effect of the k th family; sf_{ik} is the interaction between the ith site and the kth family; $rf(s)_{jk(i)}$ is the interaction between the k th family and the jth replication in the ith site; and e_{ijkl} is the random within-plot error.

Since amount of fungal DNA was based on needles from an entire family row-plot, there was a single value per family plot in each replication. Thus, the statistical model for this trait was obtained by dropping the within-plot error term (e_{ijkl}) in eq. 1.

The model for the mature data set combined over sites was

$$[2] \quad y_{ijklm} = \mu + s_i + r(s)_{j(i)} + d_k + sd_{ik} + rd(s)_{jk(i)} + f(d)_{l(k)} + sf(d)_{il(k)} + srf(d)_{ilj(k)} + e_{ijklm}$$

where y_{ijklm} is the observation on the mth tree of the lth family in the jth replication and kth set in the ith site; μ is the overall mean; s_i is the effect of the ith site; $r(s)_{j(i)}$ is the effect of the jth replication in the ith site; d_k is the effect of the kth set; sd_{ik} is the effect of the interaction between the ith site and kth set; $rd(s)_{jk(i)}$ is the effect of the interaction between the jth replication and kth set in the ith site; $f(d)_{l(k)}$ is the effect of the lth family in the kth set; $sf(d)_{il(k)}$ is the effect of the interaction between the ith site and the lth family in the kth set; $srf(d)_{ilj(k)}$ is the effect of the interaction between the ith site, the jth replication, and the lth family in the kth set; and e_{ijklm} is the random error.

To test site differences, the Satterthwaite approximation of denominator degrees of freedom was used (Satterthwaite 1946, as cited in Milliken and Johnson 1984, pp. 250–251). Tests of significance were conducted at $\alpha = 0.05$.

ANOVAs were conducted using PROC GLM (generalized linear models) of the SAS statistical package to test significance of family differences (type III sums of squares). Variance components were then estimated using the REML (restricted maximum likelihood) method of the VARCOMP procedure. REML estimates of variance components are considered more reliable than ANOVA estimates when imbalance exists in data (Swallow and Monahan 1984; Searle et al. 1992; White 1996). For estimation of variance components, all effects were treated as random effects.

Trees at the Toledo juvenile site were under attack by another fungus (*B. cinerea*). Although family differences were not found for percentage of trees with *B. cinerea*, the impact of *B. cinerea* was examined by conducting statistical analyses with and without *B. cinerea* as a covariable (scored as either present or absent). The effect of *B. cinerea* was significant for needle color (the least square mean for seedlings with *B. cinerea* = 2.01 vs. without *B. cinerea* = 1.99), needle retention (8.38 vs. 8.21) and foliage density (4.94 vs. 4.82), but not for foliage color (2.10 vs. 2.08). The family F values were also very similar when the effect of *B. cinerea* was included and not included in the model: needle color (with *B. cinerea* as covariable = 1.48 vs. without = 1.46), needle retention (1.45 vs. 1.44), foliage density (1.29 vs. 1.28), and foliage color (1.49 vs. 1.49). Thus, family variance compo-

nents were not seriously affected by *B. cinerea* infection, and its effect was not included in data analyses.

Except for PSOP and fungal DNA, all SNC variables included in this study are categorical variables, which usually violate the normality assumption of ANOVA. In fact, the distribution of plot means for these variables was not normal. However, in estimating variance components the assumption of normality is not required (Steel et al. 1997) and the *F* test is fairly robust to departures from normality, especially when sample sizes are equal (Cochran and Cox 1967; Snedecor and Cochran 1967; Neter and Wasserman 1974; Zolman 1993). Furthermore, analyses conducted with transformed (square root and natural logarithm) variables yielded variance components and heritability estimates essentially the same as those obtained with untransformed variables. Therefore, data analyses reported are those conducted using untransformed data.

Genetics of the traits and interrelationships among them were investigated by estimating additive genetic variance, individual and family narrow-sense heritabilities, and genetic and phenotypic (family mean) correlations among traits. Because wind-pollinated families in this study came from mother trees in wild stands, additive genetic variance (σ_A^2) was estimated as four times the family component of variance, assuming individuals in these families are true half-sibs. Total phenotypic variance (σ_{PI}^2) was estimated as

$$[3] \qquad \sigma^2_{PI_J} = \sigma^2_e + \sigma^2_{rf(s)} + \sigma^2_{sf} + \sigma^2_f, \qquad \text{for juvenile traits,}$$

$$[4] \qquad \sigma^2_{PI_M} = \sigma^2_e + \sigma^2_{srf(d)} + \sigma^2_{sf} + \sigma^2_f, \qquad \text{for mature traits}$$

and total phenotypic variation among family means ($\sigma_{PF}{}^2$) as

$$[5] \qquad \sigma^2_{PF_J} = \frac{\sigma^2_e}{srt} + \frac{\sigma^2_{rf(s)}}{sr} + \frac{\sigma^2_{sf}}{s} + \sigma^2_f,$$

for juvenile traits

$$[6] \qquad \sigma^2_{PF_M} = \frac{\sigma^2_e}{srt} + \frac{\sigma^2_{srf(d)}}{sr} + \frac{\sigma^2_{sf(d)}}{s} + \sigma^2_{f(d)},$$

for mature traits

The harmonic mean for the number of replications (r) and number of trees per plot (t) in eqs. 5 and 6 were produced by the GLM procedure. The number of sites (s) was two. Narrow-sense individual and family heritabilites were estimated following Falconer (1981).

Data were standardized for each trait to remove scale effects by subtracting the site mean and dividing by the site standard deviation prior to calculating genetic correlations. Additive genetic correlations (r_A) between trait pairs x and y within each age group were estimated as

$$[7] \qquad r_A = \frac{\mathrm{Cov}_f(x, y)}{\sqrt{\sigma^2_{fx}\sigma^2_{fy}}}$$

where $\mathrm{Cov}_f(x, y)$ is the family covariance between traits x and y, estimated as (Freund 1962)

$$\mathrm{Cov}_f(x, y) = \frac{\sigma^2_{f(x+y)} - \sigma^2_{fx} - \sigma^2_{fy}}{2}$$

and σ^2_{fx}, σ^2_{fy}, $\sigma^2_{f(x+y)}$ are, respectively, the family variance components for traits x and y and the sum of traits x and y. Note that family variance component for mature traits is $\sigma^2_{f(d)}$.

Type B genetic correlations (r_B) between juvenile (x) and mature (y) traits were estimated following Burdon (1977):

$$[8] \qquad r_B = \frac{r_{xy}}{\sqrt{h^2_{fx} h^2_{fy}}}$$

where r_{xy} is the family mean correlation between x and y and h^2_{fx} and h^2_{fy} are the respective estimates of family mean heritabilities of the juvenile and mature traits.

Objective 3: examining the efficiency of early selection for SNC tolerance in coastal Douglas-fir

To determine efficiency of early selection for Swiss needle cast tolerance in Douglas-fir, expected gains from direct selection, correlated response from indirect selection of correlated traits at the earlier age, and relative efficiency of early selection were calculated. Genetic gain from direct selection (G_y) was estimated as the amount of improvement in trait y expected in the progeny of a seed orchard consisting of clones of parent trees selected on the basis of the performance of their open-pollinated offspring (Namkoong 1979):

$$[9] \qquad G_y = 2ih^2_f\sqrt{\sigma^2_{PF}}$$

where i is the selection intensity. In all calculations, it was assumed that the top 10% of parent trees were selected (i = 1.755). Correlated response from family selection (CR_y) is the amount of improvement in a trait (y) when selection is applied to another trait (x) (Falconer 1981):

$$[10] \qquad \mathrm{CR}_y = 2ir_A\sqrt{h^2_{fy}\, h^2_{fx}\, \sigma^2_{PF(y)}}$$

where terms are defined as previously indicated. Efficiency of early selection was estimated as the ratio of the correlated response in a trait at the mature age when selection is applied to a comparable (or different) trait at the juvenile age (eq. 10, where y and x are the mature and juvenile traits, respectively) to the response expected from direct selection at the mature age (eq. 9).

Results

Objectives 1 and 2

The severity of SNC disease symptoms was greater in the Pleasant Valley seedling trial than in Toledo (Table 1). Variation among family means for the summer symptom traits, whether assessed in the field or in the laboratory, was also greater in the Pleasant Valley trial (data not shown). PSOP and amount of fungal DNA in sampled needles were greater at Toledo. Although the mature tests at Salal and Gordy had similar symptom severity, family variation was higher in Salal (data not shown).

Family differences were found for all traits assessed in the summer except for fungal DNA (Table 2). Fungal DNA was excluded from further analyses because it showed no significant family differences when data were combined over sites.

Among juvenile traits assessed in the fall, only needle retention and foliage density significantly differed among fam-

Table 2. Overall means (ranges in parentheses), probability of family differences, narrow-sense individual heritabilities (h_i^2) and family heritabilities (h_f^2) for summer SNC traits in juvenile (age 2) and mature (average of ages 10 and 12) trees, and age-10 height and age-13 diameter at breast height (DBH) for the mature trees.

	Juvenile				Mature			
Traits	Mean	*P* value	h_i^2	h_f^2	Mean	*P* value	h_i^2	h_f^2
Field								
Needle color	1.87 (1.50–2.41)	0.0006	0.15	0.49[a]	—	—	—	—
Foliage color	1.95 (1.50–2.38)	<0.0001	0.15	0.50	1.97 (1.61–2.42)	0.0051	0.2	0.52
Needle retention	7.85 (4.71–8.80)	<0.0001	0.23	0.60	7.19 (6.30–8.26)	0.0484	0.1	0.33[a]
Foliage density	4.64 (2.88–5.68)	<0.0001	0.25	0.64	3.81 (3.39–4.47)	<0.0001	0.4	0.73[a]
Height (m)					5.14 (4.34–5.97)	<0.0001	0.2	0.64
DBH (mm)					112.6 (93.1–128.2)	<0.0001	0.2	0.57
Laboratory								
Needle color	2.68 (2.00–3.15)	0.0178	0.06	0.26				
Needle Retention	7.35 (4.44–8.05)	0.0021	0.21	0.57				
PSOP[b]	32.67 (19.69–41.20)	0.0031	0.14	0.43				
Fungal DNA	662.9 (373.8–862.1)	0.5824	ne	ne				

Note: ne, not estimated because no significant family differences were found; "—", needle color was not measured in the mature trials (Table 1).
[a]Significant family × site interaction ($P < 0.05$).
[b]Proportion of stomata occluded with pseudothecia.

Table 3. Estimated juvenile–mature (type B) genetic correlations between Swiss needle cast severity traits and family mean correlations (in parentheses).

	Mature trait				
Juvenile trait	Foliage density	Foliage color	Needle retention	DBH[a]	Height[b]
Field					
Needle color	0.19 (0.11)	0.53 (0.27)*	0.75 (0.30)*	0.26 (0.13)	–0.07 (–0.04)
Foliage color	0.29 (0.18)	0.50 (0.25)	0.83 (0.34)*	0.20 (0.10)	–0.08 (–0.04)
Needle retention	0.25 (0.16)	0.42 (0.23)	0.75 (0.34)*	0.04 (0.02)	–0.10 (–0.06)
Foliage density	0.30 (0.21)	0.57 (0.33)*	0.63 (0.29)*	0.17 (0.10)	0.03 (0.02)
Laboratory					
Needle color	–0.34 (–0.15)	0.47 (0.17)	0.72 (0.21)	0.40 (0.14)	0.07 (0.03)
Needle retention	0.09 (0.06)	0.41 (0.22)	0.75 (0.33)*	0.09 (0.05)	0.03 (0.02)
PSOP[c]	0.09 (0.05)	0.24 (0.11)	0.25 (0.10)	–0.13 (–0.06)	–0.10 (–0.06)

Note: *, $P < 0.05$.
[a]Age-13 diameter at breast height (DBH).
[b]Height at age 10.
[c]Proportion of stomata occluded with pseudothecia.

ilies, and these family differences were less statistically significant than their summer counterparts (data not shown). Genetic correlations between the same juvenile traits assessed in the summer and fall were moderate but significant (0.57 for foliage density, 0.68 for needle retention). Because juvenile traits assessed in the fall were not significantly correlated with mature traits, the fall assessments in the juvenile trials will no longer be discussed. Significant site × family interactions were only observed for juvenile field needle color and mature needle retention and crown density. Family mean correlations between sites were moderate (mean = 0.40, range 0.28–0.60) and significant for all traits except for mature needle retention (0.25), suggesting that family rankings were similar at each site. Type B genetic correlations between sites for each trait ranged from 0.44 to 2.58 (mean = 1.04).

No family × age interaction was detected for the mature trials, so the average of the age-10 and age-12 assessments were used in the analyses.

All traits remaining in the analyses were found to be under low to moderate genetic control (Table 2). Seedling traits measured in the field had similar heritability estimates to those of the same traits measured in the laboratory (Table 2); measuring trees in the laboratory did not lead to higher heritability estimates. Among the traits assessed in mature trials, foliage density appears to be most heritable. Heritability estimates for the same traits measured in both the juvenile and mature trials were roughly similar, although foliage density maybe somewhat more heritable in mature trees than in seedlings (Table 2).

Estimated genetic correlations among SNC symptom traits measured in juvenile trees (i.e., all except PSOP), regardless of whether measured in the field or laboratory, were moderate to strong (mean $r_A = 0.82$, range 0.51–1.22), indicating that these traits are largely controlled by the same sets of genes. Except for needle and foliage color in the field, all traits assessed in the field and laboratory for juvenile trees

Table 4. Estimated genetic gains in mature traits from early selection at the juvenile age and relative efficiency of early selection (in parentheses) for trait pairs with statistically significant family mean correlations.

	Mature trait	
Juvenile trait	Foliage color	Needle retention
Field		
Needle color	0.21 (0.52)	0.19 (0.91)
Foliage color		0.21 (1.02)
Needle retention		0.21 (1.01)
Foliage density	0.26 (0.63)	0.18 (0.87)
Laboratory		
Needle retention		0.21 (0.98)

Note: Gains are for seed orchard progeny of the top 10% of parent trees selected on the basis of performance of their half-sib progeny (families).

were significantly correlated with PSOP (mean r_A = 0.72, range 0.62–0.81). Genetic correlations among SNC traits measured in mature trees were somewhat weaker (mean r_A = 0.56, range 0.42–0.76), but still strong enough to indicate that they are largely controlled by similar sets of genes.

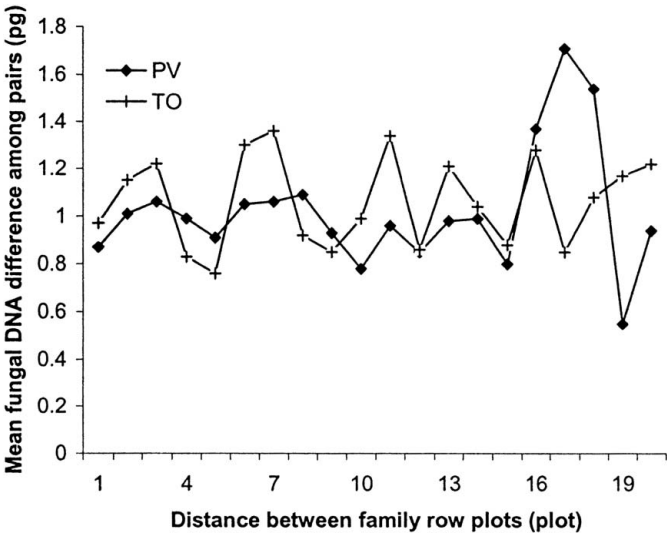
Objective 3

All juvenile traits measured in the field and needle retention in the laboratory were significantly and positively correlated with needle retention in mature trees (mean r_A = 0.74) (Table 3). Correlations of seedling traits with foliage color in mature trees were weaker and significant only for needle color (r_A = 0.53) and foliage density (r_A = 0.57). No juvenile traits were significantly associated with foliage density (mean r_A = 0.12) or growth (mean r_A = 0.06) in mature trees (Table 3). Estimates of genetic gain in tolerance of mature trees to *P. gaeumannii* were generally larger and more consistent when juvenile selections were based on field traits rather than traits measured in the laboratory (Table 4). Correlated responses from family selection indicate that selection for greener needles (or foliage) or greater needle retention (or foliage density) in seedlings will increase these traits in older trees, with expected gains nearly those achieved if selection was delayed to a mature age (i.e., selection efficiency for trait pairs with significant genetic correlations, mean = 0.80, range 0.52–1.01). Genetic correlations between SNC traits assessed in seedlings and growth traits in mature trees were not significant ($P > 0.05$, Table 3). Therefore, selection for SNC traits in seedlings is not expected to affect growth in older trees.

Discussion

Natural inoculation of seedlings that occurred in the seedling field trials of this study was both convenient and effective. Although there was no control over the amount of inoculum that seedlings received, continuous exposure to the SNC pathogen and a disease-conducive environment ensured infection and symptom development. We tried infecting Douglas-fir seedlings with *P. gaeumannii* by placing infected Douglas-fir branches over the seedlings for 1 week in a growth chamber at the Dorena Tree Improvement Center (Temel 2002). Although the seedlings were infected with *P. gaeumannii* (as evidenced by presence of *P. gaeumannii* DNA in Douglas-fir needles), the disease symptoms did not develop. Reasons for this may be threefold: the amount of spores was not sufficient, the 1 week of inoculation in the growth chamber was not long enough, or the warmer, drier climate at Dorena in contrast to the humid climate at the coast prohibited symptom development. If artificial inoculation is necessary (i.e., if natural inoculation is not possible or if the source of inoculum needs to be controlled), the ways to ensure symptom development should be determined.

Fig. 1. Variogram of fungal DNA content in one replication at each juvenile site (PV = Pleasant Valley, TO = Toledo).



As in many experiments involving airborne pathogens, heterogeneous distribution of inoculum across the plots could affect the precision and reliability of results and estimates (Jenkyn et al. 1996). Although the amount of inoculum was not determined for either age group, it is very unlikely that there was variation in inoculum distribution for two reasons. First, the spores were suspended in the air and moved freely. Second, spore release is not a one-time occurrence, but a continuous process throughout spring and summer. Thus, it is unlikely that there was plot-to-plot variation in inoculum density. Furthermore, variograms for each replication at each juvenile site were produced using plot means for fungal DNA. A variogram examines variation as a function of distance of separation and indicates whether spatial variation patterns are present in the data. An increasing trend is expected if plots closer to each other are more similar than plots spaced farther apart. No spatial pattern was observed for fungal DNA content of needles (Fig. 1). This indicates that trees closer together are as different from each other as trees spaced farther apart.

The effect the chilling treatment had on susceptibility of the seedlings is unknown, but it provided uniform bud burst, allowing all seedlings in the study to be exposed to the disease under more or less the same conditions. It is doubtful that the cold storage procedure, and the resulting uniform bud burst, altered family rankings of SNC symptoms because bud burst timing was not correlated to symptom expression in a previous study conducted in the region (Johnson 2002).

Determination of appropriate traits to use in an early testing effort was one of the main objectives. In addition to the

commonly used field assessments of SNC foliage traits (e.g., color, density, and retention), several traits were assessed in the laboratory. Laboratory measurement is more expensive than assessing traits in the field, and in this study it did not improve efficiency of early selection. Thus, scoring SNC symptom traits in the field is sufficient for early testing purposes. While severity of symptoms varied significantly among families, lack of significant family variation for fungal DNA content is consistent with the hypothesis that tolerance, rather than resistance, is the primary defense mechanism of Douglas-fir against SNC (Temel et al. 2004).

Estimates of heritabilities and genetic correlations observed in this study agree with previously published results. In a study conducted over seven 11-year-old progeny tests, heritability estimates ranged from 0.04 to 0.30 (mean = 0.17) for foliage color; from 0.16 to 0.44 (mean = 0.25) for foliage density; and from 0.03 to 0.28 (mean = 0.15) for needle retention (Johnson 2002). Genetic correlation estimates between retention (assessed on primary and secondary branch internodes for 1-year-old needles and on secondary branch internodes for 2-year-old needles) and color in Johnson (2002) is similar (mean $r_A = 0.58$), albeit slightly weaker, to estimates found in this study.

The primary goal of Douglas-fir breeding in the Pacific Northwest is to improve growth rate and wood quality traits of this species (Silen 1978), and thus early selection for any trait should not adversely affect growth and wood quality. Because the genetic correlations between SNC traits assessed in seedlings and growth traits in mature trees were not significant, selection for SNC traits in seedlings is not expected to affect growth in older trees. Foliage color and density assessed at "mature" ages have been shown to be correlated with diameter and basal area increment in this series of trials (Johnson 2002) and another series of progeny trials on the Oregon coast (Johnson 2002; Johnson et al. 2002).

Early testing is possible for improved needle retention and color because of the high genetic correlations between the two ages for these traits. Early testing can be integrated into breeding programs in a number of ways. In single-stage selection, superior genotypes are identified based on seedling performance to shorten generation intervals and increase genetic gain per unit of time. For this type of early selection to be more efficient than mature selection, in terms of gain per unit of time, the relative efficiency of early selection must be larger than the ratio of the earlier to mature generation interval. For coastal Douglas-fir, this ratio is expected to range between 0.30 and 0.75; thus, a relative efficiency of 0.50 or greater is sufficient for early selection to be more effective than mature selection (Adams et al. 2001). Needle retention and foliage color in this study met this requirement.

Multiple-stage selection is the most commonly used form of early selection in conifer breeding for disease resistance (Phelps 1977; Walkinshaw et al. 1980). In this method, genotypes that perform poorly are identified and culled at the seedling stage prior to establishment of field tests, and final selections are based on one or more additional stages of evaluation in the field. In this case, the size of field tests is reduced along with establishment costs, and statistical precision is increased by reduction of block size (Adams et al. 2001). Adams et al. (2001) estimated that 30%–40% of families could be culled with family mean correlations between juvenile and mature tests as low as 0.30 to 0.40, assuming $h_m^2 = 0.60$ and the top 20% of the original population is selected after two stages of selection. Relative efficiency of two-stage early selection (RE_{TS}) was calculated according to eq. 11 in Adams et al. (2001). In the RE_{TS} calculation for the current study, we assumed 10% of the original population is selected after two stages of selection (i.e., 80% at the first stage and 12.5% at the second stage), and found two-stage early selection would be 92% and 100% as efficient as mature selection for needle retention and foliage color, respectively.

Early selection for SNC tolerance can shorten the breeding cycle, but it is not practical under current breeding strategies for Douglas-fir because selection for the most important traits (i.e., growth and wood density) should be made at age 12–15 to optimize gain (Johnson et al. 1997). Early testing for SNC tolerance can still serve in two ways under current breeding strategies in the region. First, new families to be progeny tested can be screened for SNC tolerance, and families that perform poorly can be eliminated before testing. Second, early testing for SNC tolerance might be a means of quickly screening very large numbers of families already selected for other traits, so that the most SNC-tolerant families can be identified for immediate use in seed orchards.

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