# Genetic Studies Involving Swiss Meedle Cast





Figure 1. Parent tree locations for families studied in the geographic variation study.

Randy Johnson, USDA-FS; Fatih Temel, OSU; and Keith Jayawickrama, OSU

## Abstract

Three studies were analyzed this year that examined genetic aspects of Swiss needle cast (SNC) tolerance. Families sampled across the Siuslaw National forest showed differences in foliage health traits, but very little of the variation could be explained by environmental or climatic conditions at the parent tree location. Five test sites of the Nehalem series of progeny trials were measured for DBH. The best families were continuing to have reasonable basal area increments, implying that genetic improvement can produce stock that could offset

> the 30% volume growth reduction predicted in moderatelyimpacted SNC areas. A third study suggested that Douglas-fir families do not appear to increase 1<sup>st</sup>-year needle production in response to needle loss and families may differ in the amount of foliage they produce for a given branch diameter.

> Three genetics studies looking at aspects of tolerance to SNC were completed this year. These studies examined the genetic variation patterns of SNC tolerance over the landscape, the effectiveness of genetically improving tolerance, and whether families differ in their ability to respond to SNC with increased needle production. The first study was supported by SNC Cooperative funding because it was done in conjunction with the early testing study. The second study was funded by the Oregon Department of Forestry and the TRASK metacooperative and the last study was established by the USFS PNW Research Station.

## Study 1 – Geographic Variation in Swiss Needle Cast Tolerance of Douglas-fir in the Siuslaw National Forest

### **Methods and Materials**

Genetics of Swiss needle cast (SNC) tolerance and relationships between climatic and geographic variables at parent tree locations and SNC tolerance were investigated in 152 windpollinated Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) families originating from the central coast of

Table 1.	Means and ranges of t	1e 14 geographic and	d climatic variables
considered	in the analyses.		

Variable	Mean (Range)
Distance to the Pacific Ocean (km)	15.20 (1.00 - 48.90)
Elevation (m)	331 (25 – 667)
Latitude (UTM)	49398.57 (48854 —
50173)	
Slope (%)	20.32 (0 - 65.25)
Aspect (cosine)	-0.06 (-0.65 – 0.51)
Aspect (sine)	-0.03 (-0.45 – 0.46)
June precipitation (mm)	78.26 (52.13 – 114.14)
June average maximum temperature (°C)	20.30 (16.86 - 23.25)
June average minimum temperature (°C)	8.53 (7.19 – 9.26)
June potential evapotranspiration rate	130.60 (85.38 – 156.36)
November precipitation (mm)	367.80 (290.63 - 433.18)
November average maximum temperature (°C)	12.26 (9.82 – 13.49)
November average minimum temperature (°C)	4.30 (2.07 – 5.32)
November potential evapotranspiration rate	34.95 (0.00 - 42.48)

Oregon in the Siuslaw National Forest (Figure 1). Families were planted on two test sites (Toledo and Pleasant Valley, Figure 1) and naturally inoculated with Phaeocryptopus gaeumannii spores that came from the surrounding infected stands (see Temel and Johnson 2001, Temel 2002). The two-year-old progeny were visually assessed for foliage greenness (at both needle [needle color] and crown [foliage color] levels) and proportion of retained foliage (at both single internode [needle retention] and crown [foliage density] levels). Because the two color traits and the two retention traits are controlled virtually by the same sets of genes, as evidenced by genetic correlations close to 1, geographic variation was investigated only in foliage color and foliage density because they were slightly more heritable than needle color and needle retention.

Foliage traits were regressed on a total of 6 geographic and 8 climatic variables at mother-tree locations that might be related to variation in SNC severity. The environmenatal factors considered (Table 1) were those that have been shown to play important roles in variation in severity of SNC symptoms (Michaels and Chastagner 1984,

Capitano 1999, Manter *et al.* 2000, Rosso and Hansen 1999).

Climatic variables were estimated for mother tree locations (UTM) using two different models. Mean minimum and maximum daily temperatures (°C) for each month and mean monthly precipitation (mm) were obtained from PRISM (Precipitation-elevation Regressions on Independent Slopes Model) (Daly et al. 1994). Monthly rates of evaporation, potential evapotranspiration and woody transpiration were obtained from the MAPSS (Mapped Atmosphere-Plant-Soil System) (Neilson 1995) model.

#### Results

Results of ANOVA indicated significant family differences for all foliage traits. There were no

significant family-by-site interactions for any of the traits, indicating that the family rankings were consistent from site to site. Foliage traits were weakly heritable, with individual narrow-sense heritability estimates ranging from 0.11 to 0.20. Family mean heritabilities were moderate and ranged from 0.44 to 0.56. Heritability estimates were similar for related traits (i.e., needle color and foliage color, needle retention and foliage density).

Foliage density was not found to be significantly associated with any of the geography and climate variables. A small amount of variation in foliage color (14.5%) could be explained by a significant (best fitting) model containing a subset of the geography and climate variables (Table 2).

The limited evidence reported here suggests that a portion of variation in foliage color is associated with the geography of mother tree locations. Lack of such evidence for foliage density suggests that color is more responsive to natural selection

#### Table 2. Regression equation for foliage color.

Variable	Parameter	Partial R <sup>2</sup>	P-value	
	Estimate			
Intercept	1.8889	_	<0.0001	
Distance to the				
Pacific Ocean	0.0179	0.0296	0.0053	
(Distance to the				
Pacific Ocean) <sup>2</sup>	-5.0163 * 10 <sup>-10</sup>	0.0340	0.0029	
Cosine of Aspect	-24.2088	0.0240	0.0118	
Latitude-by-Cosine				
of Aspect	0.0005	0.0238	0.0123	
(Elevation) <sup>2</sup>	-5.1643 * 10 <sup>-7</sup>	0.0332	0.0032	
Model (C	o = 5.72):	0.1446	0.0003	

pressures of SNC. Within the Siuslaw National Forest there is not a strong tendency for climate and geography to influence the SNC tolerance of parent tree collections. This result could be because SNC has not applied significant selection pressure in the past.

## Study 2 – Nehalem Breeding Program – Age-17 Assessment

#### **Methods and Materials**

In 1995, five progeny test sites were assessed for height, DBH, foliage color, crown density and needle retention. These age-11 results were reported in the 1998 annual report (Johnson and Temel 1998). In 1998, an additional DBH assessment was made on three of the five sites and examined 120 of the 400 total families (reported in the 1999 SNCC Annual Report, Johnson and Temel 1999). During the fall of 2001, all 400 families were assessed for DBH on three sites, and the remaining two sites were assessed in the summer of 2002. From these data we were able to analyze 6-year basal area increment. If one defines tolerance as continued growth in the presence of disease, then basal area over this time period represents the trait of choice to obtain SNC tolerance.

Heritabilities (h<sup>2</sup>) were estimated for all traits that were assessed (see Johnson 2002 for statistical methods). Heritability represents the proportion of the observed variation that is controlled by a tree's genes; the remainder is controlled by environmental effects. Genetic and family-mean correlations of the age-11 traits with basal area increments were also estimated. Genetic correlations are a result of the same genes (or tightly linked genes) affecting two different traits.

The efficiency of cooperative breeding programs was examined by looking at the performance of selected families in the progeny test. Selection was based on (1) using only age-11 data to select for subsequent basal area growth (primarily) and foliage health or (2) including age 11 to 17 basal area increment to the age-11 selection criteria. This represents selection based at two different ages. Trait scores were first generated for each age-11 trait using a selection index procedure (Johnson 1997) that weighs family-site means according to the site's family mean heritability and average genetic correlation with the other sites. The age-17 data weighted the family-site means only by the site's family heritability (all sites were well correlated and almost equally correlated with one another). The family scores for each trait were standardized to mean of 0 and a standard deviation of 10. An overall index score for each family was generated by multiplying the standardized trait scores by what we felt to be appropriate values. The weightings were as follows:

- Age-11 index =  $(2 \times DBH)$  + Ht +  $(0.5 \times Color)$  +  $(0.5 \times Crown Den$ sity) +  $(0.3^*Retention)$
- Age-17 index = (2×BA inc) + DBH + Ht + (0.5×Color) + (0.5×Cr Den) + (0.3\*Reten)

where all traits are age-11 except for basal area increment from age 11 to 17 (BA inc).

#### Results

Heritabilities for the age-17 traits were larger that those for the age-11 traits and growth traits typically had larger heritabilites than the foliage traits (Table 3). The age-17 DBH and basal area increment data were well correlated across sites. The family mean correlations averaged 0.49 (ranged from 0.42 to 0.57)and the genetic correlations averaged 0.86 (0.76 to 1.00).

The age-11 growth traits were better correlated with subsequent basal area growth than were the foliage traits (Table 4). Of the foliage traits, color and crown density were better correlated with subsequent basal area growth than retention. Crown density had the largest heritability of the foliage traits.

Table 3. Narrow-sense heritabilities for age-11 color, crown density, needle retention, height, DBH and subsequent basal area increment on five progeny test sites of Douglas-fir (standard errors in parentheses).

Site	Color	Crown density	Needle retention	Height	Age-11 DBH	Age-17 DBH	Basal area increment
Acey Crk.	0.04(0.03)	0.23 <i>(0.04)</i>	0.16 <i>(0.04)</i>	0.38 <i>(0.05)</i>	0.40 <i>(0.05)</i>	0.49 <i>(0.05)</i>	0.47 <i>(0.05)</i>
Coal Crk	0.10 <i>(0.03)</i>	0.19 <i>(0.04)</i>	0.13 <i>(0.04)</i>	0.32 <i>(0.05)</i>	0.32 <i>(0.05)</i>	0.40 <i>(0.05)</i>	0.39(0.04)
Cole Mt.	0.10 <i>(0.03)</i>	0.16 <i>(0.04)</i>	0.34 <i>(0.05)</i>	0.30 <i>(0.04)</i>	0.27(0.04)	0.47 <i>(0.07)</i>	0.49(0.08)
Davis Crk.	0.27 (0.04)	0.28 <i>(0.04)</i>	0.28 <i>(0.04)</i>	0.33 <i>(0.04)</i>	0.32(0.04)	0.41 <i>(0.04)</i>	0.41(0.04)
Slick Rock	0.20 <i>(0.04)</i>	0.22(0.04)	0.20 <i>(0.04)</i>	0.37 <i>(0.05)</i>	0.32 <i>(0.04)</i>	0.46 <i>(0.04)</i>	0.46 <i>(0.04)</i>

Table 4. Family mean and genetic correlations of age-
11 traits with subsequent basal area growth.

Age-11 trait	Family-mean correlation	Genetic correlation
DBH	0.80	0.90
Height	0.73	0.76
Color	0.47	0.59
Crown density	0.49	0.55
Needle retention	0.20	0.25

The top 40 families selected for tolerance with the age-11 index averaged 24% more basal area growth than the population mean (Table 5). Selection using the age-17 index resulted in 27% more basal area than the population mean. More gain was obtained by using the age-17 data, but this gain would be realized 6 years later than one would get if selection was made at age 11. Gainper-year of breeding was greater when selecting at age 11 than waiting until age-17..

Percentage gains from using the parents in a seed orchard would be greater than the family means from the progeny trials because genetic gain from a seed orchard is equal to:

Table 5. Means for total population and for top 10% (40 families) selected with age-11 and a combination of age-11 and –17 data. Percentage increase reported in parentheses.

	Population	Top 10% Index	Top 10%
Traits	mean	Age-II	Age-17 Index
Age 11-17 Basal area (mm²)	14334	17783 (24.1%)	18283 (27.5%)
Age 11-17 DBH increment (mm/yr)	10.1	11.6 (14.6%)	11.8 (17.2%)
Age 17 DBH (mm)	170.3	188.7 (10.8%)	190.6 (13.9%)
Age-11 DBH (mm)	107.7	117.0 (8.6%)	117.2 (8.8%)
Age-11 height (cm)	752	795 (5.7%)	797 (6.0%)
Color	1.90	1. <b>94 (2</b> .1%)	1.95 (2.6%)
Crown density	3.85	3.98 (3.4%)	4.00 (3.9%)
Retention	4.73	5.10 (7.8%)	5.05 (6.8%)

Genetic gain =  $2 \times (\text{family mean heritability}) \times (\text{Selection differential})$ 

Estimated genetic gain for basal area increment using the age-17 index would be  $2 \times 0.825 \times 27.5\% = 45\%$ . Estimated genetic gain for annual diameter increment of the top 40 families was  $2 \times 0.815 \times 17\%=27.7\%$ ; which translates to a diameter growth rate of 12.9 mm/yr; or about four rings per inch. We suggest this to be an acceptable growth rate for Douglas-fir growing under moderate SNC-pressure (average of 2 years needles).

## Study 3 – Family Responses to Foliage Abuse

It has been noticed that certain trees appear to have more needles than others, but actual needle retention is the same. This study attempted to look at the family differences in a tree's ability to put on foliage and whether it changes when 2<sup>nd</sup> year foliage experience occluding of their stomates and premature casting.

### **Methods and Materials**

A subset of 20 families from the early-testing study were grown in raised beds at the Forest Science Lab in Corvallis. In an attempt to simulate the occluding of stomates (what pseudothesia do to needles), the undersides of needles were sprayed with paint (1<sup>st</sup> year) or polyurethane (2<sup>nd</sup> and 3<sup>rd</sup> years) just prior to bud burst. Four trees per family were planted across a nursery bed. Each nursery bed contained two replications, i.e. two blocks, each with 20 4-tree family rows. In each bed, the trees on one side of the bed were sprayed and other half left as controls. The timetable of events was as follows:

Summer 1998	Seeds sown in greenhouse
Spring 1999	Foliage sprayed with white-wash green- house paint prior to bud burst, seedlings transplanted to raised beds
Fall 1999	Heights measured
Spring 2000	Foliage sprayed with exterior grade poly- urethane prior to bud burst
Fall 2000	Heights and Diameter measured
Spring 2001	Foliage sprayed with exterior grade poly- urethane prior to bud burst
Fall 2001	Heights and basal diameter measured
Winter 2001	Foliage sampled and measured in two beds (4 of 6 replications).

In the winter of 2001, foliage was sampled from two 2-yr-old branches from each tree in two of the three beds. Basal diameter of each branch was measured and ovendry weight of the 1<sup>st</sup> and 2<sup>nd</sup> year needles were obtained for the two branches combined. From this information foliage mass per unit basal area for 1<sup>st</sup>-year needles and the 2<sup>nd</sup>year needles was calculated.

#### Results

The sprayed trees were shorter, had smaller diameters and fewer 2ndyear needles per unit basal area than the unsprayed trees (Table 6). This reduction could be due to either occluded stomates, chemical damage, or poisoning from the spray treatments. Family height differences were evident at the age-3 measurement (p=0.0623), but had disappeared by age 4 (p=0.3658). No familv differences were evident for diameter growth. Families did show differences for the ratio of 1st-year needles to branch basal area. For all traits there were no family-by-treatment differences, implying that families performed the same in each treatment.

These results suggest that trees are not responding to needle loss by producing more 1<sup>st</sup>-year foliage because the 1<sup>st</sup>-year foliage to basal area ratio did not increase in the sprayed trees. Second-year foliage was reduced by the spraying, as expected. Because families differ in the amount of foliage they produce per unit of branch basal area, there may be a possibility that the families that produce more foliage per branch basal area demonstrate more SNC tolerance. However, there was no significant correlation found between the ratio and age-10 growth and foliage data from these same families in field tests. Lack of correlation could be the result of none being present or the limited amount of data avail-

Table 6. Mean heights, diameters and foliage to basal area ratios for trees sprayed with polyurethane prior to bud burst and the unsprayed controls, and level of significance for the difference.

Trait	Sprayed trees	Unsprayed controls	Level of significance
Height (age 2)	47.25 cm	49.75 cm	0.0373
Height (age 3)	55.33 cm	60.90 cm	< 0.001
Height (age 4)	101.11 cm	114.35 cm	< 0.001
Diameter (age 3)	12.38 mm	12.57 mm	0.0279
Diameter (age 4)	17.84 mm	19.10 mm	< 0.001
Foliage ratio — 1 <sup>st</sup> yr needles	0.331 g/mm²	0.335 g/mm²	0.0969
Foliage ratio — 2 <sup>nd</sup> yr needles	0.072 g/mm <sup>2</sup>	0.082 g/mm <sup>2</sup>	0.0047

able for estimating ratios (12 trees from 20 families).

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