AN ABSTRACT OF THE THESIS OF

Fatih Temel for the degree of Doctor of Philosophy in Forest Science presented on June 5, 2002. Title: Early Testing of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) for Swiss Needle Cast Tolerance.

Abstract Approved: Signature redacted for privacy.

George R. Johnson W. Thomas Adams

The relationship between the level of *Phaeocryptopus gaeumannii* (Rohde) Petrak colonization and severity of Swiss needle cast (SNC) symptoms, the possibility of early testing of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) for SNC tolerance, and geographic variation in coastal Oregon with respect to SNC tolerance were investigated.

Comparisons between the amount of *P. gaeumannii* DNA and SNC symptom severity (i.e., degree of yellowing or loss of needles) of 6 wind-pollinated Douglas-fir families (two families for each of three disease severity groups) in two progeny test plantations revealed that there were no immune Douglas-fir trees; infection and colonization of Douglas-fir needles by *P. gaeumannii* occurred at similar rates in the different disease severity groups. The presence of significant differences in symptom severity among the groups, therefore indicated that tolerance, rather than resistance, is responsible for the observed variation in disease symptoms. The degree to which a tree retains its last four growing season's needles appears to be a useful measure for assessing tolerance to SNC.

Trees from 55 wind-pollinated Douglas-fir families were assessed for SNC symptoms in two 2-year-old (juvenile) and two 10- and 12-year-old (mature) progeny tests to investigate the genetics of SNC tolerance in Douglas-fir and the possibility of early testing. Natural inoculation was found to be suitable for early testing purposes. Traits related to SNC tolerance (i.e., needle retention, needle color, foliage color and foliage density) were under low to moderate genetic control

($0.11 \leq h_i^2 \leq 0.37$) at both ages. Moderate genetic correlations between juvenile and mature trees for needle color ($r_B^2 = 0.53$) and needle retention ($r_B^2 = 0.75$) make these traits suitable for early selection. Early selection for needle retention in seedlings is expected to be as efficient as selection in older trees for improving needle retention in older trees, while early selection for needle color is estimated to be 52% as efficient as later selection for needle color.

Two-year-old progeny of 152 wind-pollinated Douglas-fir families originating from the Siuslaw National Forest were assessed for severity of SNC symptoms in two seedling trials to investigate genetics of SNC tolerance and relationships between SNC tolerance of families with climatic and geographic variables of mother tree locations. The locations ranged between 25 and 667 m (mean = 331 m) in elevation and between 1 and 48.9 km (mean = 15.2 km) in distance to the Pacific Ocean. The southernmost and the northernmost mother tree locations were north of Florence and south of Pleasant Valley, respectively. Tolerance to SNC was found to be weakly heritable. Despite a wide range among families in severity of SNC symptoms, no significant relationships were observed between needle retention and geographic and climatic variables at mother tree locations. Foliage color in SNC infected trees, however, was significantly associated, although weakly ($R^2 = 0.14$), with geography; with greenest foliage in families originating from lower elevations, southerly aspects and midway in the west-east transect across the Coast Range Mountains (i.e., greenest at about 20 km from the coast). The lack of strong patterns of SNC tolerance may be because geographic variation in SNC infection is limited within this region or because there has not yet been sufficient selection pressure on Douglas-fir for patterns to eveolve.

Early Testing of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) for Swiss Needle Cast Tolerance

by
Fatih Temel

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented June 5, 2002
Commencement June 2003

Doctor of Philosophy thesis of Fatih Temel presented on June 5, 2002.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Fatih Temel, Author

ACKNOWLEDGMENTS

First and foremost I am most indebted and grateful to the people of my country, Turkey, for providing this rare opportunity for me to pursue my Ph.D. in the United States.

I would like to thank my advisors Drs. Randy Johnson and Tom Adams for their guidance and patience throughout my studies. Special thanks to Drs. Jeffrey Stone, David Birkes, Shawn Mehlenbacher and Peter List for serving on my committee, and invaluable suggestions and input.

I received help from many people during my studies. I would like to thank Lee Riley, Joseph Linn, Richard Sniezko and personnel of the Dorena Tree Improvement Center for taking great care of my seedlings and providing facilities for artificial inoculations.

Members of the Forest Genetics team at USFS Pacific Northwest Research Station were of great assistance. Nancy Mandel, Frank Sorensen, Sara Lipow, and Brad St.Clair provided practical solutions to many questions I had for them. Technicians Al Sugano, Jeffrey Riddle, Richard Miles, and Sean Woods provided assistance in site preparation and data collection. Ken Vance-Borland provided climatic and geographic data. I thank you all very much.

I wish to thank Loretta Winton and Wendy Sutton for their assistance in the plant pathology laboratory.

I thank student workers Leah Fickes and Stacy Sandstrom for the great job they did on sample collection, symptom assessment and data entry. I also thank fellow graduate students Oktay Yıldız and Santiago Martinez Gonzales for their assistance in sample collection.

I thank the members of Swiss Needle Cast Cooperative for funding this study. Special thanks to Simpson Timber Company for providing land and planting crew for establishing the test sites, and to The Timber Company (presently Plum Creek Co.) for providing cold storage for seedlings.

($0.11 \leq h_i^2 \leq 0.37$) at both ages. Moderate genetic correlations between juvenile and mature trees for needle color ($r_B^2 = 0.53$) and needle retention ($r_B^2 = 0.75$) make these traits suitable for early selection. Early selection for needle retention in seedlings is expected to be as efficient as selection in older trees for improving needle retention in older trees, while early selection for needle color is estimated to be 52% as efficient as later selection for needle color.

Two-year-old progeny of 152 wind-pollinated Douglas-fir families originating from the Siuslaw National Forest were assessed for severity of SNC symptoms in two seedling trials to investigate genetics of SNC tolerance and relationships between SNC tolerance of families with climatic and geographic variables of mother tree locations. The locations ranged between 25 and 667 m (mean = 331 m) in elevation and between 1 and 48.9 km (mean = 15.2 km) in distance to the Pacific Ocean. The southernmost and the northernmost mother tree locations were north of Florence and south of Pleasant Valley, respectively. Tolerance to SNC was found to be weakly heritable. Despite a wide range among families in severity of SNC symptoms, no significant relationships were observed between needle retention and geographic and climatic variables at mother tree locations. Foliage color in SNC infected trees, however, was significantly associated, although weakly ($R^2 = 0.14$), with geography; with greenest foliage in families originating from lower elevations, southerly aspects and midway in the west-east transect across the Coast Range Mountains (i.e., greenest at about 20 km from the coast). The lack of strong patterns of SNC tolerance may be because geographic variation in SNC infection is limited within this region or because there has not yet been sufficient selection pressure on Douglas-fir for patterns to evolve.

CONTRIBUTION OF AUTHORS

Dr. Jeffrey Stone was instrumental in initiation of the study reported in Chapter 2 and provided consultation throughout the study.

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DEDICATION

This thesis is dedicated to the loving memory of my grandmother, Ayşe Özyurt.

Early Testing of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) for Swiss Needle Cast Tolerance

Chapter 1

Introduction

Swiss needle cast (SNC) is a fungal foliage disease of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) caused by *Phaeocryptopus gaeumannii* (Rohde) Petrak. Although native to the Pacific Northwest, the disease was first described early in the twentieth century in Douglas-fir plantations in Switzerland, hence the name (Gäumann 1930). Later reports of damaging effects of SNC came from New Zealand (Hood and Kershaw 1975), Australia (Hood 1997), Europe (Boyce 1940, Merkle 1951) and northeastern United States (Morton and Patton 1970, Merrill and Longenecker 1973) where Douglas-fir was introduced as an exotic.

In coastal Oregon, airborne spores of the fungus infect newly emerging Douglas-fir needles through their stomata starting in late spring. Infection and colonization of inter-cellular spaces of the needles continue throughout the summer. By mid-winter fungal fruiting bodies (pseudothecia) emerge from stomatal openings impairing their ability to control gas exchange and water loss. Infected needles discolor and are shed prematurely resulting in sparse yellowish tree crowns and reduction in tree growth. In heavily affected areas trees lose all but the current year's needles.

Although the disease and the fungus causing it have been known to foresters in the Pacific Northwest region of the United States since the beginning of the twentieth century (Boyce 1940), it has not been considered damaging in the

region until recently (Kanaskie *et al.* 1996, Hansen *et al.* 2000). SNC was first reported to be a problem in off-site Douglas-fir Christmas tree plantations (Hadfield and Douglas 1982). Since then the incidence of the disease has increased in both natural and plantation Douglas-fir stands (Hansen *et al.* 2000). Annual aerial surveys indicate that approximately 300,000 acres of Douglas-fir are affected by SNC in coastal Oregon (Annual Report 2000, Swiss Needle Cast Cooperative, Oregon State University, Corvallis, Oregon). Reduction in annual stem volume growth due to SNC was estimated to be 23% in affected areas and up to 52% in heavily affected stands (Maguire *et al.* 2002).

Widespread occurrence of the disease prompted research to better understand genetics of tolerance and/or resistance in Douglas-fir, the biology of the pest, its distribution and interactions with the environment, and to develop disease prevention methods (Swiss Needle Cast Cooperative). As a result we now have a better understanding of the disease (Capitano 1999, Hansen *et al.* 2000), its impacts on tree physiology (Manter *et al.* 2000) and growth (Maguire *et al.* 2002), the biology of *P. gaeumannii* (Winton 2001), and better tools and methods to assess the severity of the disease (Manter *et al.* 2001, Winton 2001, Maguire *et al.* 2002).

Genetic variation in Douglas-fir with respect to SNC symptom severity was reported for full-sib families in a Christmas tree plantation (Nelson *et al.* 1989), among clones growing in Romania (Blada 1988), and at the provenance level in British Columbia (McDermott and Robinson 1989). Johnson (2002) observed weak genetic control over symptom severity in half-sib progeny test plantations in coastal Oregon. Presence of genetic variation in symptom severity suggested that Douglas-fir can be bred for resistance or tolerance to SNC.

In order to reforest sites damaged by a series of forest fires in the 1930's and 1940's near Tillamook, Oregon, forest managers were forced to use whatever Douglas-fir seed was available at the time; most of the seeds came from inland sources because of unreliable seed years on the coast. Use of inappropriate seed sources is cited as one of the potential reasons why a formerly benign parasite has

"suddenly" become a problematic pest in coastal Oregon (Hansen *et al.* 2000). Although there is limited information suggesting that seed sources from more inland and drier portions of western Oregon are more susceptible to SNC than seed sources from higher rainfall areas nearer to the coast (McDermott and Robinson 1989, Johnson and Temel 1999 SNCC Annual Report), further investigation of the geographic variation in symptom severity is needed. If there are significant geographical patterns in SNC symptom severity, it might be necessary to re-evaluate the current Douglas-fir seed transfer guidelines.

The following three main objectives are addressed in this thesis.

Objective 1: Relationship between SNC symptom severity and levels of P. gaeumannii infection. To our knowledge no individual Douglas-fir trees are completely immune to this disease. It is possible, however, to find adjacent infected trees that display different levels of symptom severity. Are these differences due to varying levels of resistance to *P. gaeumannii* (i.e., do some Douglas-fir trees have less infection and fungal growth in their needles?) or to varying levels of tolerance to infestation (i.e., are some trees better able to maintain foliage and good growth in the presence of the fungus)? Understanding the mechanisms by which Douglas-fir deals with SNC and the traits which best represent these mechanisms would be very useful for breeding efforts.

Objective 2: Early testing of Douglas-fir. Early testing is the process in forest tree breeding where trees are selected after being grown at close spacing in a greenhouse, growth chamber, or nursery for one or two years (Lambeth 1983). It has three main advantages: (1) the length of the breeding cycle can be reduced, thus increasing genetic gain per unit of time; (2) efficiency of progeny testing can be increased by culling susceptible families before field testing; and, (3) selection intensity can be increased by testing larger number of families (Wu 1998, 1999). If an early selection procedure can be developed, it can be especially useful in breeding Douglas-fir against SNC, because it would be much more convenient to assess symptoms in small seedlings than older trees. For this purpose a reliable

method for inoculating seedlings is required. In addition, traits that are at least moderately heritable and under the control of similar sets of genes in both seedlings and at the final selection stage (i.e., older, usually ≤ 15 years in Douglas-fir) are needed.

Objective 3: Geographic variation in Douglas-fir with respect to SNC symptom severity. Swiss needle cast has apparently been present in the Pacific Northwest for a long time. If SNC has applied variable selection pressure on Douglas-fir in habitats conducive to infection (e.g. cool, moist areas on coast) and habitats less conducive to infection (e.g. dry, warm areas away from coast), then degree of resistance or tolerance to this disease can be expected to vary with geography and climatic conditions in the region. Determining geographical and climatic factors associated with symptom severity may lead to re-evaluation of the current seed transfer guidelines in coastal Oregon.

There are five chapters in this thesis. Chapters 2, 3 and 4 address the three main objectives listed above in order, the last chapter is a general conclusion and summary of the research in this thesis. Appendices include data and additional pertinent information.

Chapter 2

The Relationship Between Swiss Needle Cast Symptom Severity and Level of *Phaeocryptopus gaeumannii* (Rohde) Petrak Colonization in Coastal Douglas-Fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco).

2.1 Abstract

A total of 108 15-year-old trees belonging to 6 wind-pollinated Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) families (2 families from each of 3 disease severity groups- light [i.e., good needle retention, dense dark green foliage and good growth], severe [i.e., poor needle retention, very sparse yellowish foliage and inferior growth], and moderate [i.e., somewhat between the light and severe groups]) were assessed for Swiss needle cast (SNC) symptoms and *Phaeocryptopus gaeumannii* infection both in the field and in the laboratory, in order to understand the relationship between SNC symptom severity and actual level of infection, and to evaluate traits that might be used in breeding Douglas-fir for SNC tolerance. Needle retention, needle color, foliage density and foliage color were visually assessed on trees in the field. In addition to needle retention and color, amount of fungal biomass (as determined by proportion of stomata occluded with pseudothecia or by amount of pathogen DNA in sampled needles) and several needle size variables were assessed on one- and two-year-old internodes in the laboratory. Trees in different disease severity groupings were similar with respect to amount of fungus in their needles, yet needle retention, foliage color and foliage density were significantly better in the light group than the others. The only statistically significant ($P < 0.05$) relationship between amount of fungus in needles and SNC symptoms was between amount of fungal DNA in one-year-old needles and average needle retention over the last four growing seasons. Average needle retention decreased with increased amount of pathogen DNA in the light group. This relationship was reversed in the severe group and there was no relationship in the moderate group. Because amount of fungal DNA did not differ significantly among the groups, differences in symptom severity were attributed to tolerance, not resistance. Visual scoring of individual trees for average retention needles over the past four growing seasons could be used to effectively assess for SNC tolerance in Douglas-fir breeding programs.

2.2 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 it has been introduced as an exotic (Morton and Patton 1970, Merrill and Longenecker 1973, Hood and Kershaw 1975). 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 20th century (Boyce 1940), SNC was not considered damaging until recently (Hansen *et al.* 2000). In Douglas-fir plantations severely effected by SNC, up to 23% reduction in annual growth rates have been reported (Maguire *et al.* 2002).

The major symptoms of the disease include foliage discoloration (yellowing) and premature casting 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 loss control) by fungal fruiting bodies, pseudothecia (Manter *et al.* 2000). Airborne spores of the fungus infect newly emerging Douglas-fir needles in the spring with colonization of intercellular spaces of needles continuing throughout summer (Capitano 1999). Pseudothecia begin to emerge from stomatal openings by winter. Only newly emerged needles are susceptible to infection by *P. gaeumannii* spores (Hansen *et al.* 2000), but hyphae, germinated from pseudothecia, continue to grow on needle surfaces and infect additional stomata as needles age (Capitano 1999).

To our knowledge no individual Douglas-fir trees are immune to this pathogen. Nevertheless, there are significant differences in severity of disease symptoms and growth, even between trees that occupy the same environs. Part of this variation is attributed to genetic variation in Douglas-fir (Johnson 2002). The mechanism involved with this variation is not known. Is it resistance, i.e., do "better" looking trees actually inhibit the level of infection, or is it tolerance, i.e.,

are "better" looking trees somehow able to continue to grow in the presence of the disease (Agrios 2000)?

Douglas-fir breeding programs in the region have focused mainly on improving growth and wood quality (Silen 1978, Temel and Adams 2000). Reports of significant growth losses due to SNC, however, prompted immediate interest in the possibility of breeding Douglas-fir against SNC, along with other research on the disease and the pathogen (Swiss Needle Cast Cooperative, College of Forestry, Oregon State University, Corvallis, Oregon). Presence of genetic variation in symptom severity indicates Douglas-fir can, in fact, be bred for improved growth in the presence of this disease (Johnson 2002).

A disease symptom is a visible or otherwise measurable adverse changes in a plant, produced in reaction to infection by a microorganism or to an unfavorable 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. Methods of assessing colonization include proportion of stomata occluded with pseudothecia (Hansen *et al.* 2000) and amount of fungal biomass in the needles determined by quantitative PCR (Polymerase Chain Reaction, Winton 2001) or ergosterol (Manter *et al.* 2001) methods. Symptoms of SNC are degree of yellowing of needles and extent of defoliation (casting). Several visually scored variables have been employed to assess severity of the SNC symptoms. These are foliage color, needle retention and foliage density (Johnson 2002). Foliage color is degree of yellowing in a tree's foliage. While needle retention is proportion of needles retained in a given internode, foliage density measures density of foliage in the entire crown. Which, if any, of these traits best represent resistance/tolerance to SNC, however, is not known. Furthermore, in any breeding effort, in order to improve efficiency and to reduce cost of selection, the number of traits included should be minimized (Zobel

and Talbert 1984). Douglas-fir breeders in the region would greatly benefit in their efforts to breed Douglas-fir against SNC, if the resistance/tolerance mechanisms are understood and one or more traits that actually represents this mechanism is determined.

This study was undertaken to (1) better understand Douglas-fir's mechanisms underlying the observed variation in SNC symptom severity among different trees, and (2) to compare the relationship between level of *P. gaeumannii* infection and symptom severity. A total of 108 trees from 6 wind-pollinated Douglas-fir families were assessed in two progeny test plantations in coastal Oregon, both in the field and in the laboratory for SNC signs and symptoms.

2.3 Material and Methods

2.3.1 Plant Material

The six open-pollinated Douglas-fir families were identified from two sets of 40 families; two families per disease severity group- light, moderate and severe. Disease severity rating for each family was based on data from two previous SNC and tree growth field assessments conducted at 5 sites in 1995 and 3 sites in 1998. Two sites assessed in both 1995 and 1998 were the Acey Creek and Coal Creek progeny test plantations, near Tillamook, OR, where the effects of the disease are pronounced (Table 2.1). An index value for each family was calculated by summing un-weighted standardized values (mean = 0, standard deviation = 10) of each variable (needle retention, foliage color, foliage density and diameter at breast height [1.37 m, DBH], see Table 2.2 for definitions and assessment methods) over 80 families. The standardized values were used because of the scale differences among the variables. Then, the families were listed in a descending order according to their index values and the two top (very light SNC symptoms, or

"light"), two middle (somewhat severe SNC symptoms, or "moderate") and two bottom (very severe SNC symptoms, or "severe") families in the list were chosen for this study. The plant material included extremely well performing (i.e., good growth, good needle retention and dense foliage) and extremely poor performing (i.e., inferior growth, poor needle retention and sparse foliage) trees under disease pressure, because any relationship between *P. gaeumannii* infection and SNC symptom severity would be most pronounced in these families. Two moderately affected families were included to serve as an intermediate reference point between the extremes.

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 (Table 2.1). 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 randomized complete block design with 3 replications, within which each family was represented by a 4-tree non-contiguous plot at the time of planting. Due to 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.

2.3.2 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). First, a tree belonging to one

Table 2.1. Location information on progeny test plantations.

	Progeny Test Plantation	
	Acey Creek	**Coal Creek**
Latitude	45° 45' 35" N	45° 46' 11" N
Longitude	123° 47' 25" W	123° 51' 34" W
Elevation (m)	204	67
Distance to the Pacific Ocean (km)	6	4
Planting year	1986	1986
General aspect	E	SE

of the families was found in the plantation and its DBH, foliage color and foliage density were assessed (see Table 2.2 for description of traits and measurement methods). Then, a south-facing branch in the 4th whorl from the top of the tree was cut with a pole prunner. The sampled branch was selected from south side of the upper crown where disease symptoms are more pronounced (Hansen *et al.* 2000). Second flushing, or lammas growth, is a common occurrence in coastal Douglas-fir, resulting in multiple whorls in single growing season (Kaya *et al.* 1994). Because second flushing occurs frequently in young Douglas-fir growing in the moist, mild conditions of coastal Oregon, care was taken in determining the whorl that was produced as a result of initial bud flush at the beginning of the growing season. When on the ground, the diameter of this branch was measured at its base and needle color and needle retention were scored on internodes of the lateral branches representing foliage produced in each of the past 4 growing seasons (i.e., 1996 to 1999) (Figure 2.1).

Three 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 ("S" for south tip of branch, "E" for east and "W" for

west). These small bags were then placed in larger bags separately for each growing season. The sampled tree's identity and sample's age (1 for 1999 and 2 for 1998 foliage) were recorded on an index card and placed in the bag with the internodes. The same information was also written on the bag with a permanent marker. Samples were kept in an ice chest in the field and transported to USDA Forest Service Pacific Northwest Research Station, Forest Genetics Group's freezer in Corvallis, OR. The samples were kept frozen until the laboratory assessments were conducted.

For laboratory assessments, only samples from east and west sides of the sample branch (i.e., laterals) were used because disease symptoms were most prominent in these branchlets. After pulling a bag from the freezer, 5 randomly selected needles from the each internode (total 10 needles) were bulked and placed in a screw cap micro-centrifuge tube (Island Scientific, IS-522) along with two solid glass beads (Fisher Scientific, 11-312C) for DNA extractions. Tubes were marked with an identification number that corresponded to the site, replication, family and tree information. These samples were kept in the freezer until DNA extraction. Later, the internodes were lined on black cardboard after thawing. Needle color was assessed visually comparing each samples' color with a color photo of needles that illustrated each of the 4 distinct color groups (Figure 2.2).

Finally, 25 needles from the east and west internodes of each sampled branch (50 needles total) were randomly selected and weighed. Ten of the weighed needles (5 from each of the east and west branchlets) were placed side by side on a 10 cm by 20 cm clear glass plate (lower side of needles facing up) with petioles in the same direction. The remaining 40 needles were also placed on the same glass plate and spaced so that none of them contacted with each other. These needles were fixed in their positions by putting another glass plate on them and holding the

Table 2.2. Traits assessed and assessment methods.

Traits (abbreviation) **Field**	Assessment method
Needle retention (FNR)	Visually scored from 0 (0-10% retention) to 9 (91-100% retention) on the sampled branch for each of the past 4 growing seasons (i.e., for 1996, 97, 98 and 99).
Needle color (FNC)	Visually scored from 1 (yellow) to 3 (dark green) on the sampled branch for each of the past 4 growing seasons.
Foliage color (FFC)	Visual color score of overall tree crown from 1 (yellow) to 3 (dark green).
Foliage density (FD)	Visual score of foliage density over entire tree crown from 1 (sparse) to 6 (dense).
DBH (mm)	Diameter of the tree measured at the breast height.
Branch diameter (mm) (BRD)	Diameter of the sampled branch measured at the base.
Laboratory	These traits were assessed for 1999 and 1998 needle cohorts separately.
Needle retention (%) (LNR)	Average needle retention on the east and the west sample internodes obtained by dividing total number of needles by total number of needle scars.
Needle color (LNC)	Average visual color score of east and west samples scored from 1(yellow) to 4 (dark green) by comparing the samples with color photos of sample foliage.
Percent stomata occluded with pseudothecia (%) (PSP)	Proportion of stomata occluded with pseudothecia on the 10 needles, based on 100 stomata per needle.
Fungal DNA (pg) (DNA)	Amount of fungal DNA obtained by quantitative PCR.
Needle length (mm) (NL)	Average length of 10 needles.

Table 2.2 (Continued)

Fresh weight (g) (FRW)	Fresh weight of 50 needles.
Dry weight (g) (DRW)	Air-dried weight of 50 needles.
Internode length (cm) (IL)	Average length of the east and the west sample internodes.
Projected needle specific area (cm^2/g) (PNSA)	Obtained by dividing projected area to fresh needle weight.
Moisture content (g) (MOIST)	Difference between fresh weight and dry weight.

two plates together with clips (Figure 2.3). Each plate was labeled with sample information.

Midpoints of each of the 10 identified needles between the plates were marked with a pen. Then the plates were placed under a dissecting microscope with needle petioles toward the observer. On the second stomata row at the right hand side of the needle midrib, the central 100 stomata were examined (1000 stomata for per sample) for presence or absence of fungal pseudothecia, to estimate the proportion of stomata occluded with fungal fruiting bodies (Hansen *et al.* 2000). Lengths of these 10 needles were also measured.

Length of each sample internode was measured in millimeters, and number of needles present on the needles and total number of needle scars were counted. Needle retention was then determined by dividing number of needles to potential number of needles.

The projected area of needles placed between the glass plates was measured by video image recorder and AgVision software (Decagon Devices, Inc., Pullman, WA) and the needles were placed in small paper envelopes for drying. Air-dry weights of needles were recorded.

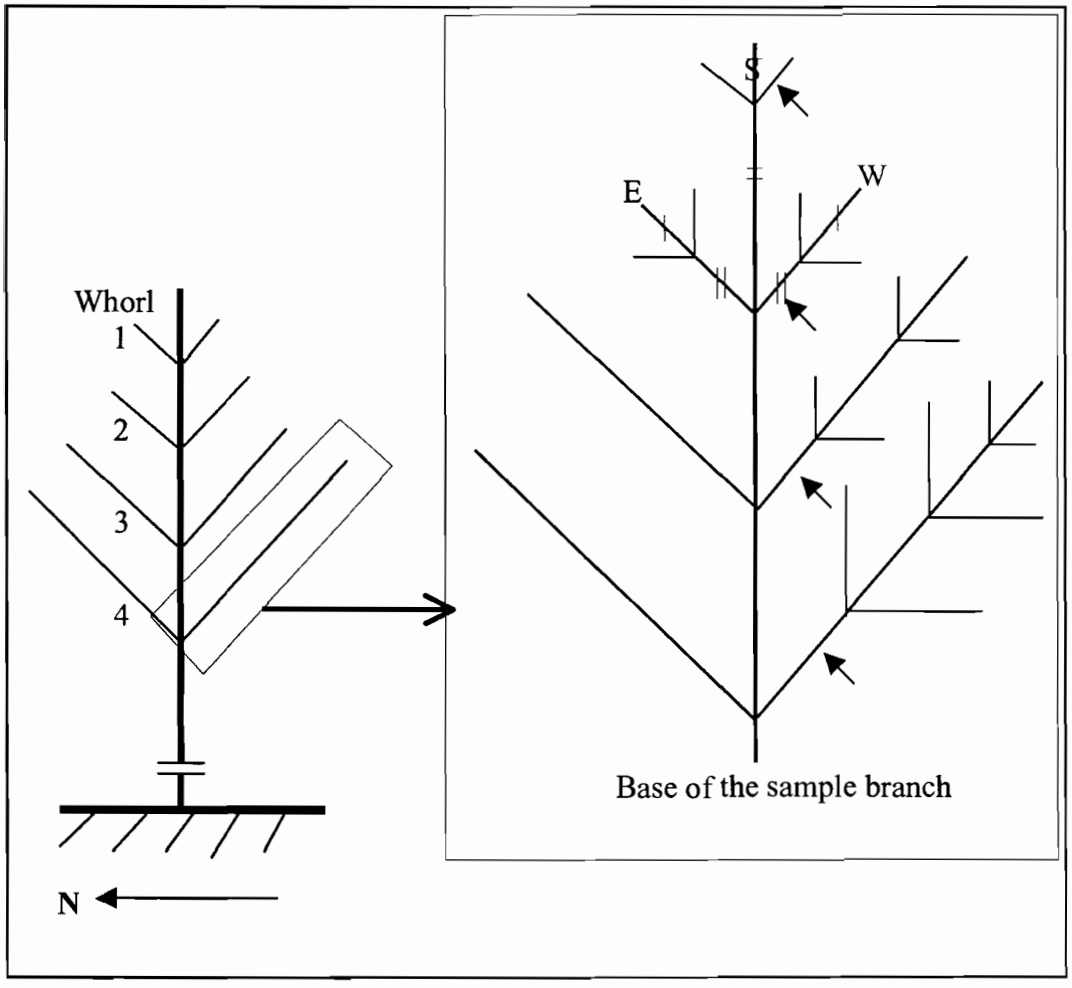


Figure 2.1. Illustration of the sampling procedure. Sampled internodes are marked (| = 1999 cohort, || = 1998 cohort; arrows indicate internodes scored for needle retention and color in the field).

Total genomic DNA was extracted from 10 needles placed in micro-centrifuge tubes. The needles were frozen in liquid nitrogen and pulverized in a Mini-Beadbeater (Biospec Products, Bartlesville, OK) for 30 s at 4200 rpm. After pulverization, samples were incubated in 1.5 ml extraction buffer (2% CTAB

Figure 2.2. Photograph of the four representative needle color groups used in the laboratory assessment of needle color.

(cetyltrimethylammonium bromide), 100 mM Tris, pH 8.0, 20 mM Na_2EDTA pH 8.0, 1.4 M NaCl, 1% polyvinylpolypyrrolidone, 0.1 % 2-mercaptoethanol) at 65 °C for 2 hours. The DNA was purified in 24:1 chloroform:isoamyl alcohol, precipitated from the aqueous phase by the addition of isopropanol, washed in 70% ethanol, and re-suspended in 1 ml TE (5 mM Tris, pH 8.0, 0.5 mM Na_2EDTA), and real-time PCR was employed to quantify *P. gaeumannii* DNA (Winton *et al.* 2002).

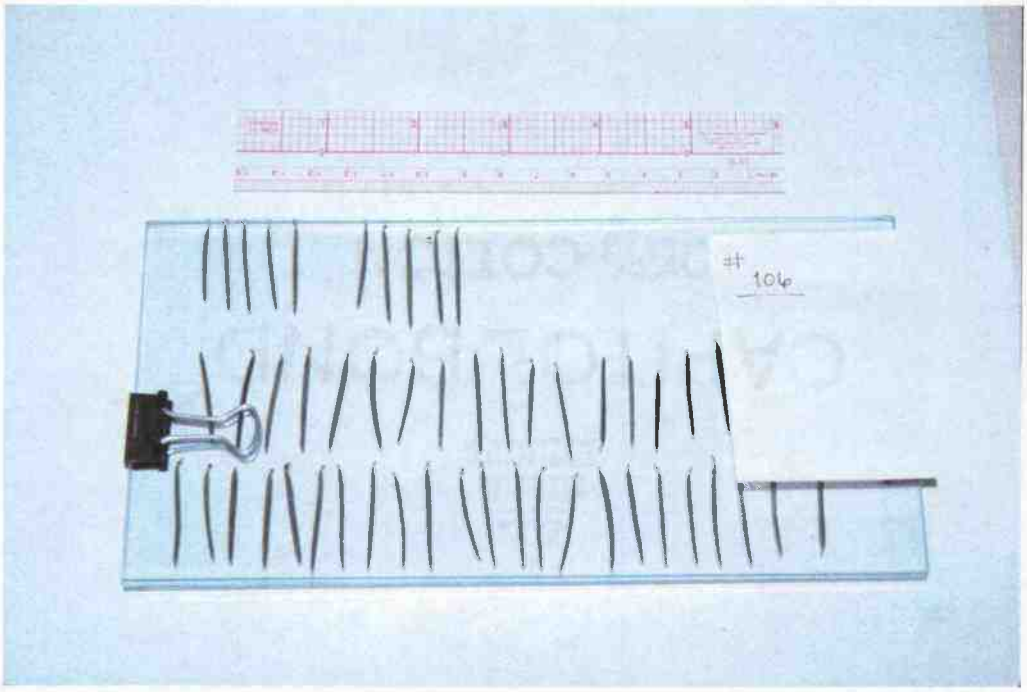


Figure 2.3. Glass plates with needles between them. Each glass plate included 50 needles from two branchlets (E and W) for each age (1998 and 1999) from a sampled branch. Needles in the upper left corner are separated into two groups of 5 one group from the east and one from the west branchlet. The remaining needles are a mixture of the two aspects (20 from each), with location of needles, by aspect, not retained.

2.3.3 Data Analysis

Data collected in this study can be divided into three groups: (1) data collected in the field, and (2) data collected in the laboratory from 1999 cohort (1-year-old) samples and (3) from 1998 cohort (2-year-old) samples. Needle retention and needle color assessed in the field were averaged over the last 4 growing seasons and these average values were used throughout the analyses. For needle color, internode length and needle retention assessed in the laboratory, data were averaged for the east and west samples. All other variables were assessed on bulked needles from the two samples. Preliminary investigations indicated that

amount of fungal DNA and percent stomata occluded with pseudothecia violated the normality and constant variance assumptions of analysis of variance (ANOVA) and were transformed to natural logarithms before analyses (Steel and Torrie 1980). All reported means, however, are before transformation, unless otherwise noted. Differences were deemed significant at $\alpha = 0.05$. Since, the main goal in this study was to investigate extremes of disease severity and since there were no significant differences between the families in any of the disease severity groups, analyses were conducted to test differences among the disease severity groups, not among families. The data combined over the two sites were used because the error variances were consistent from site to site for each variable (Appendix A).

Objective 1: Determining resistance/tolerance mechanism. In order to investigate amount of variation and differences among the disease severity groups, variable means and ranges were calculated and analyses of variance (ANOVA) were carried out for all variables. ANOVAs were conducted using PROC GLM (generalized linear models) of the SAS statistical package (SAS Institute Inc. 1990) according to following mixed model:

$$y_{ijkl} = \mu + s_i + r_{j(i)} + \tau_k + sd_{ik} + rd(s)_{jk(i)} + e_{ijkl},$$

where; y_{ijkl} is the value of the l_{th} tree of the k_{th} disease severity group in the j_{th} replication of the i_{th} site; μ is the overall mean; s_i is the effect of the i_{th} site, $E(s_i) = 0$, $Var(s_i) = \sigma_s^2$; $r_{j(i)}$ is the effect of the j_{th} replication at the i_{th} site, $E(r_{j(i)}) = 0$, $Var(r_{j(i)}) = \sigma_r^2$; τ_k is the fixed effect of the k_{th} disease severity group, $\sum_{k=1}^{3} \tau_k = 0$; sd_{ik} is the interaction between the i_{th} site and the k_{th} disease severity group, $E(sd_{ik}) = 0$, $Var(sd_{ik}) = \sigma_{sd}^2$; $rd(s)_{jk(i)}$ is the interaction between the k_{th} disease severity group and the j_{th} replication in the i_{th} site, $E(rd(s)_{jk(i)}) = 0$, $Var(rd(s)_{jk(i)}) = \sigma_{rd(s)}^2$; and e_{ijkl} is the random error, $E(e_{ijkl}) = 0$, $Var(e_{ijkl}) = \sigma_e^2$.

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.

$$y_{jklm} = \mu + r_j + f_k + a_l + fa_{kl} + e_{jklm}$$

where; y_{jklm} is the value of the l_{th} age in the m_{th} tree of the k_{th} family in the j_{th} replication; μ is the overall mean; r_j is the effect of the j_{th} replication, $E(r_j) = 0$, $Var(r_j) = \sigma_r^2$; f_k is the effect of the k_{th} family, $E(f_k) = 0$, $Var(f_k) = \sigma_f^2$; a_l is the effect of the l_{th} age, $E(a_l) = 0$, $Var(a_l) = \sigma_a^2$; and e_{jklm} is the random error, $E(e_{jklm}) = 0$, $Var(e_{jklm}) = \sigma_e^2$.

Objective 2: Investigating the relationship between level of infection and symptom severity. In order to investigate factors influencing symptom severity regression analysis was employed. The ANOVA indicated that the symptom variables that significantly different among the three groups in the field were needle retention (FNR), foliage color (FFC) and foliage density (FD). These three variables were used as the response variables in regression analyses. Explanatory variables were fungal biomass (DNA or PSP), GROUP (light, moderate and severe), site and replication. First, 6 full models were run for each dependent variable using PROC GLM of SAS (SAS Institute Inc. 1990). In each of the 6 full models one of the following fungal biomass variables (DNA99, DNA98, MDNA (average DNA of 1999 and 1998), PSP99, PSP98 or MPSP (average PSP of 1999 and 1998) was regressed against one of the three symptom variables. Disease severity group, site and replication were also included as categorical variables in regressions. Form of the full model was as follows;

$Y=\beta_0 + \beta_1$*(Fungal Biomass)* $+$ *GROUP*$_k + \beta_3$*(Fungal Biomass-by-GROUP interaction)* $+$ *SITE*$_i$ $+$ *REPLICATIONj.*

After each run, significant terms in each model were determined and regressions were repeated using only significant terms and interactions among them.

2.4 Results and Discussion

Overall, severity of the disease symptoms was similar at both sites. Symptoms were slightly more pronounced in Acey Creek but the sites were significantly different only for FNR (Table 2.3). Tree growth and needle size were greater at Acey Creek than at Coal Creek, but the differences between sites were not statistically significant.

Objective 1. Pseudothecia and fungal DNA were present in all sampled needles, indicating that all trees included in this study were infected by *P. gaeumannii*. Both PSP and DNA were highest in the severe group and the lowest in the light group (Table 2.4), but differences among groups were not statistically significant for these traits. As expected the severity of disease symptoms increased from the light group to the severe group. Group differences were, however, only significant for FNR, FFC, FD and LNR98 (P-values = 0.02, 0.01, .005, and 0.01, respectively).

Fungal biomass was greater in two-year-old (1998) needles than in one-year-old (1999) needles in all disease severity groups (Table 2.4). This is in agreement with the results obtained in previous studies (Capitano 1999, SNCC Annual Reports). Neither the retention nor the color of one-year-old needles differed significantly among the groups. Retention (LNR) of two-year-old needles was higher in the light group than in the other two groups and the difference was statistically significant (Table 2.4). These results indicate that variations in symptom expression are due to tolerance, not resistance, because while all trees were infected and had similar amounts of fungus in their needles the family groups displayed different levels symptom severity.

Table 2.3. Overall means of variables included in this study. [1)]

Trait	Acey Creek		Coal Creek		Overall	
Field						
FNR	4.05		4.43		4.24*	
FNC	1.50		1.51		1.51	
FFC	2.39		2.35		2.37*	
FD	3.67*		3.31*		3.49*	
DBH (mm)	160.67*		155.06*		157.86*	
BRD (mm)	19.19		18.67		18.93	
Laboratory	1999	1998	1999	1998	1999	1998
LNR	75.97[†]	58.91	79.54[†]	60.39	77.58	59.65
LNC	3.43[†]	3.18	3.54[†]	2.61*	3.48	2.89
PSP (%)	3.69[†]	10.71	2.56[†]	8.42	3.13	9.57
DNA (pg)	111.05[†]	302.06	90.22[†]	264.24	100.64	283.15
NL (mm)	27.12	27.76	25.85	27.08*	26.48	27.42
FRW (g)	0.58[†]	0.65	0.50*[†]	0.61	0.54	0.63*
DRW (g)	0.30[†]	0.35	0.25[†]	0.31	0.28	0.33
IL (cm)	15.53[†]	17.90	12.30*[†]	16.14	13.91	17.02
PNSA	27.97	26.35	30.52[†]	26.73	29.25	26.54
MOIS (g)	0.28	0.30	0.25[†]	0.30	0.26	0.30

[1)] See Table 2.2 for explanation of the abbreviations. All values before transformation. [*)] Significant family differences (P = 0.05). [†)] Significant differences between 1999 and 1998 (P = 0.05).

Table 2.4. Means of variables included in this study for each disease severity group (see Table 2.2 for explanation of the abbreviations). See Appendix A for individual family means in each group at each site.

Trait	Light		Moderate		Severe	
Field						
FNR*	4.88		4.05		3.81	
FNC	1.63		1.51		1.38	
FFC*	2.78		2.30		2.03	
FD*	4.39		3.17		2.92	
DBH (mm)	178.75		152.08		142.75	
BRD (mm)	20.77		18.83		17.18	
Laboratory	1999	1998	1999	1998	1999	1998
LNR	78.50	66.81*	75.91	54.80	78.87	57.33
LNC	3.64	3.21	3.54	2.72	3.28	2.75
PSP (%)	2.63	9.18	3.18	9.88	3.57	9.65
DNA (pg)	91.33	257.77	100.74	289.76	109.84	301.92
NL (mm)	27.50	28.62	26.62	27.53	25.33	26.13
FRW (g)	0.60*	0.71	0.54	0.64	0.47	0.56
DRW (g)	0.32	0.37*	0.27	0.34	0.24	0.28
IL (cm)	14.65	18.07	13.63	17.17	13.46	15.83
PNSA	28.61	25.91	29.21	26.65	29.91	27.07
MOIS (g)	0.28*	0.34	0.27	0.30	0.23	0.28

*) Significant differences among disease severity group. §) See table 2.2 for explanation of abbreviations.

Objective 2: The only significant relationships found between infection level and severity of disease symptoms were for FNR and DNA99. For all other disease symptoms and disease signs, significant terms in regression models included either the intercept alone or the intercept with group (Table 2.5).

Table 2.5. Regression equations.*

DNA99 as the fungal biomass variable
FNR=2.87994 + 0.00321(*DNA99*) + 1.42137(*GROUP*) – 0.22351(*DNA99* GROUP*)
FFC=1.62037 + 0.375(*GROUP*)
FD=2.01852 + 0.73611(*GROUP*)
DNA98 as the fungal biomass variable
FNR=4.24306
FFC=1.62037 + 0.375(*GROUP*)
FD=3.49074
MDNA (average of DNA99 and DNA99 as the fungal biomass variable
FNR=3.17361 + 0.53472(*GROUP*)
FFC=1.62037 + 0.375(*GROUP*)
FD=2.01852 + 0.73611(*GROUP*)
PSP99 as the fungal biomass variable
FNR=3.17361 + 0.53472(*GROUP*)
FFC=1.62037 + 0.375(*GROUP*)
FD=2.01852 + 0.73611(*GROUP*)
PSP98 as the fungal biomass variable
FNR=4.24306
FFC=1.62037 + 0.375(*GROUP*)
FD=3.49074
MPSP (average of PSP99 and PSP98) as the fungal biomass variable
FNR=4.24306
FFC=1.62037 + 0.375(*GROUP*)
FD=2.01852 + 0.73611(*GROUP*)

*All terms significant at p = 0.05 except for DNA99 in FNR. DNA99, DNA98, MDNA, PSP99, PSP98 and MPSP are log transformed.

Average needle retention (FNR) decreased with increased DNA99 in the best performing (i.e. light group) families. This relationship was reversed in severely affected (i.e. severe group) families. In the intermediate families needle retention was not affected by fungal biomass (Figure 2.4).

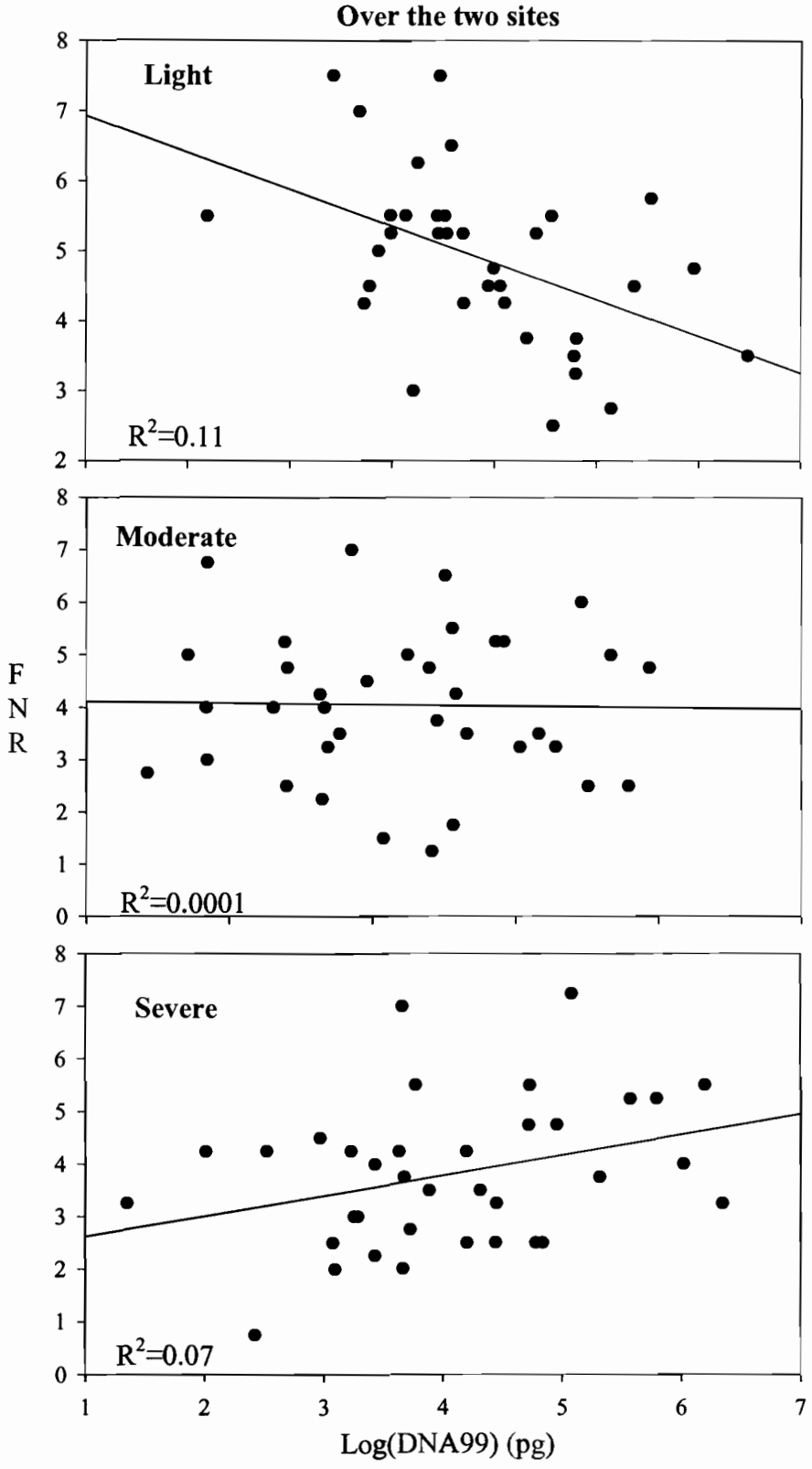
There are several hypotheses on factors influencing leaf life spans in plants (Chabot and Hicks 1982). Many of these hypotheses are based on cost and benefit relationship in producing and maintaining foliage (Johnson and Tieszen 1976, Miller and Mooney 1976). In general, when the initial cost of foliage 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 (Manter *et al.* 2000), reducing leaf productivity per unit of time. Since no statistically significant differences were found among different disease severity groups with respect to fungal biomass, and since needle retention either decreased or increased depending on initial amount of fungal biomass in the light and severe groups, respectively, one can argue that the most tolerant families produce photosynthetically very efficient, inexpensive foliage and shed infected needles as soon as maintenance cost exceeded benefits gained from them. In the severe group needle production cost is probably high with low photosynthesis rate. Therefore, infected needles in the severe group are kept longer than in the light group to compensate for these disadvantages.

Laboratory assessments of SNC symptoms are much more labor intensive than the field assessments. Needle morphology and branch variables were assessed in this study to determine if needle or branch size is related to SNC symptoms. Lack of any significant relationships between these traits and SNC symptom severity indicates that morphology traits cannot be used in predicting the severity susceptibility to SNC.

For all SNC symptoms assessed in the laboratory, significant but weak correlations exist with the similar traits assessed in the field (Table 2.6). While among the laboratory traits only retention of 2-year-old needles significantly

Figure 2.4. Relationship between log(fungal DNA content in 1999 needles) and field needle retention. See Table 2.5 for regression equation and Table 2.4 for descriptions of terms.

Figure 2.4.


Over the two sites
Light
R²=0.11
Moderate
R²=0.0001
Severe
R²=0.07
FNR
Log(DNA99) (pg)

differed among the groups, FNR, FFC and FD assessed in the field were significantly different among the groups. Therefore, more practical field assessments of SNC traits are preferable to more labor intensive laboratory assessments.

Since FNR is the only trait that appears to be related to level of infection, this trait would best indicate the tolerance of a tree under examination. In the guidelines developed by Kanaskie *et al.* (1999 SNCC Annual Report), and in previous SNC assessments, retention of one and two-year-old needles are employed for assessing SNC severity (SNCC Annual Reports). Results in this study indicate that while needle retention was higher in all needle cohorts in the light group than in the other two groups, the differences among the groups are significant in two-year-old needles in the laboratory assessments and three-year-old needles in the field assessments. Assessing the last four years' needle retention provides a better picture in terms of tolerance than assessing one or two-year-old needles. Needle cohorts older than 4 years do not need to be assessed because in the infected trees these needles are completely shed (Randy Johnson, pers. comm.).

In this study needle retention was assessed on a branch located in the upper crown of the trees. In assessing a large number of trees for breeding purposes, cutting a branch is neither desirable nor possible. Thus, the method used in this study needs to be modified. Trees can either be assessed at a younger age when their upper crowns are still visible from the ground or on lower branches in older trees.

Table 2.6. Correlations between SNC symptoms assessed in the field that differ significantly among disease severity groups and SNC symptoms and signs assessed in the laboratory.

Laboratory Assessments	Field assessments		
	FNR	FFC	FD
1999 needles			
LNC	0.29	0.21	NS
LNR	0.35	NS*	0.26
PSP	NS	NS	NS
DNA	NS	NS	NS
1998 needles			
LNC	0.28	0.24	0.32
LNR	0.64	0.37	0.35
PSP	NS	NS	NS
DNA	NS	NS	NS

*) Not significant.

2.5 Conclusions

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 pathogen seems to infect the needles and grow in infected needles at a similar pace even in the seemingly most tolerant Douglas-fir families. The tolerance mechanism seems to be associated with reduced photosynthetic rates of colonized needles. The trees with least symptoms, or more

tolerant, are least susceptible to losing their needles overall, i.e., have highest mean FNR, but are most sensitive to needle lose with increased level of infection. This way, a needle is shed when cost of maintaining it exceeds the benefits from it. 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.

None of the variables assessed in the laboratory proved to be a better replacement for similar variables assessed in the field. Visual assessments of foliage traits seem to be sufficient in determining tolerance. Among the foliage traits assessed in the field, average retention of last four growing seasons' foliage appears to be the most suitable trait for further SNC assessments because, this trait is both significantly different among the family groups and is the only trait found to be associated with actual level of fungal colonization of the needles.

The method employed in this study for assessing needle retention is not suitable when large numbers of trees need to be assessed, such as in breeding programs. One solution to this problem would be to assess trees at a younger age while their crowns can easily be observed from the ground. In this case, assessments at a younger age should be good predictors of family performances at the selection age ($\leq$ 15-year for Douglas-fir in coastal Oregon).

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Chapter 3

Early Testing of Coastal Douglas-Fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) for Swiss Needle Cast Tolerance

3.1 Abstract

Swiss needle cast (SNC), caused by *Phaeocryptopus gaeumannii* (Rohde) Petrak, has recently become a problem in coastal Oregon where it is associated with significant growth loss in Douglas-fir stands. The possibility of early testing of coastal Douglas-fir for SNC tolerance was investigated using 55 wind-pollinated Douglas-fir families. Progeny of the 55 families were visually evaluated in two seedling trials (at age 2, i.e., juvenile) and in two 10- 12-year-old (i.e., mature) progeny tests for foliage traits related to severity of SNC symptoms (foliage color: degree of yellowing over entire crown, foliage density: density of entire crown, needle retention: proportion of retained needles in a single internode, and needle color: degree of yellowing of needles in a single internode). Further laboratory assessments were made on needle samples collected from the juvenile tests in an attempt to find potential traits useful for in early testing. Genetics of the traits and reliability of early selection were determined. Natural inoculation was found sufficient to infect seedlings. None of the traits assessed in the laboratory were suitable for early testing purposes. Foliage traits were under low to moderate genetic control at both ages; individual tree narrow-sense heritability estimates ranged from 0.11 to 0.37 (mean = 0.19). Foliage traits were under the control of the same sets of genes as evidenced by genetic correlations of 0.56 to 0.95 (mean = 0.75) in juvenile tests and of 0.42 to 0.76 (mean = 0.56) in mature tests. Moderate genetic correlations between juvenile and mature tests for needle color (0.53) and for needle retention (0.75) make these traits suitable for early selection. Early selection is expected to be as efficient as mature selection for needle retention and 52% as efficient for needle color.

3.2 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 infect newly emerging Douglas-fir needles in the spring through their stomata. Infection and colonization of the needles by the fungus continue throughout summer. In coastal Oregon, fungal fruiting bodies (i.e., pseudothecia) emerge from the stomata by winter obstructing the ability of needles to control water loss and gas exchange (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 mid summer (Michaels and Chastagner 1984, Stone *et al.* 1999 Swiss Needle Cast Cooperative Annual Report, College of Forestry, Oregon State University, Corvallis, Oregon). Infected needles discolor and are cast prematurely (Hansen *et al.* 2000) resulting in significant 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 1900's (Boyce 1940), it had not been considered damaging. 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 and wood density. In the late 1980's, 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 23% growth reduction in diseased Douglas-fir stands in coastal Oregon.

All Douglas-fir trees are susceptible to infection by *P. gaeumannii* and the fungus seems to grow in the needles at a similar pace after the infection, but severity of the disease symptoms varies from tree to tree (Chapter 2 of this thesis). No evidence of quantitative resistance or immunity has been found.

A few reports on genetic variation in tolerance of Douglas-fir to SNC, and heritability of 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 family 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 9 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 in Germany (Stephan 1997). Johnson (2002) found that needle retention, crown density and foliage color differed among 505 wind-pollinated Douglas-fir families in 7 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 these traits 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.

Due to their biology, the length of the breeding cycle is very long in forest trees. Any procedure that reduces the length would be very useful. Early testing/screening is one such procedure that is a useful tool in forest tree breeding. Trees are selected after being grown at close spacing in a greenhouse, growth chamber, or nursery for one or two years (Lambeth 1983). Early testing has three main advantages: (1) length of the breeding cycle can be reduced thus increasing genetic gain per unit of time; (2) efficiency of progeny testing can be increased by culling the most susceptible families before field planting; and, (3) selection intensity can be increased by testing a larger numbers of families (Wu 1998, 1999).

An early testing procedure would be especially useful in the case of breeding Douglas-fir for SNC tolerance. The disease symptoms can be assessed

easily and probably more reliably in small trees than in larger trees. There could be new traits that would predict tolerance in small trees but are expensive to measure in older trees. Since small trees can be tested in a small area, a more uniform environment (e.g. disease pressure) could be provided. Genetic gains can be maximized using one of many early selection schemes (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*. Three artificial inoculation methods are presently available. Two of these methods mimic natural inoculation, where seedlings are placed under an infected Douglas-fir stand or under infected Douglas-fir branches in a growth chamber (Manter *et al.* 2000). The third method involves spraying seedlings with distilled water in which fine particles of mycelium are suspended (Winton 2001).

Once the seedlings are successfully inoculated and disease symptoms developed, 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. In addition, assessment methods should be practical and inexpensive. While the infection takes place primarily in May and June, colonization of needles happens throughout the year and severity of disease symptoms increases with time. Thus, appropriate timing of assessments may be critical.

There are several traits employed to assess severity of SNC based on the disease symptoms and signs. Symptoms are host's reactions to invasion by a pest, whereas signs are visible and/or quantifiable existence of the pest organism on the host (Agrios 2000). Yellowness and density of foliage, and proportion of retained needles are frequently employed foliage traits in SNC assessments (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 PCR (polymerase chain reaction) (Winton *et al.* 2002) or by ergosterol analysis (Manter *et al.* 2001) are common signs of SNC used in assessments. While the sign traits are continuous variables, subjective visual scoring (categorical) assessment methods are employed to assess foliage traits. Although visual assessments are quick and less labor intensive, they are subjective and thus their value is questionable.

The main goal of this study was to develop an early testing procedure to breed Douglas-fir for SNC tolerance. This involved the determination of an appropriate inoculation method and suitable SNC tolerance assessment traits, evaluation of quantitative genetics of these traits, and the feasibility of early selection for SNC tolerance.

3.3 Material and Methods

3.3.1 Plant Material

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 (Figure 3.1). Parent tree locations ranged in elevation from 162 m to 442 m. Individual trees from the 55 families were assessed at two 2-year-old (i.e., juvenile, Toledo and Pleasant Valley) and two 12-year-old (i.e., mature, Gordy and Salal) progeny tests. In the context of early selection "mature" would usually refer to rotation age. While 12-years is only about one-quarter of 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 al.* 1997)

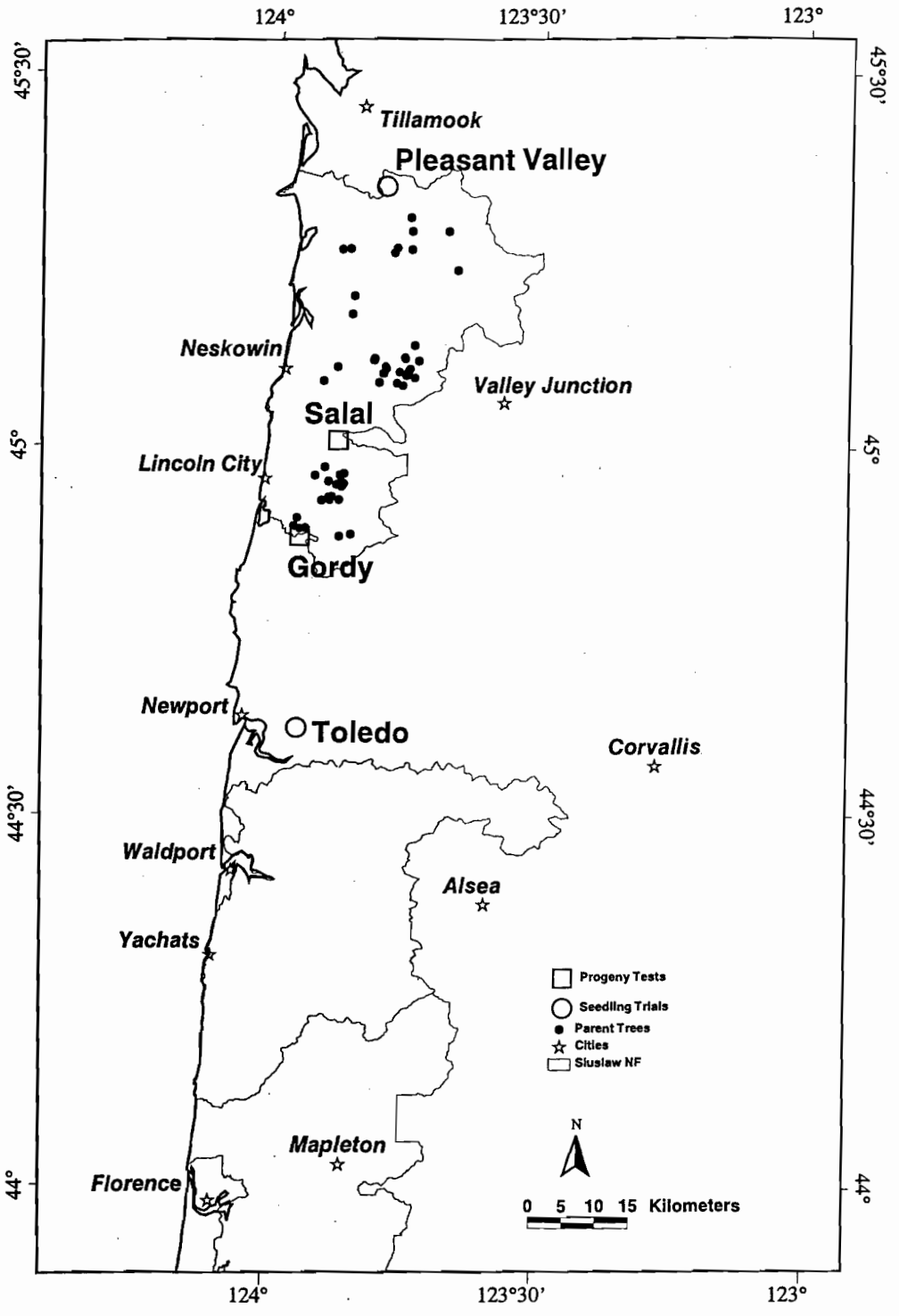

Figure 3.1. Locations of 55 parent trees, seedling trials and progeny tests in coastal Oregon.

Therefore, for simplicity, these 12-year-old plantations will be called "mature" test sites.

Plant material to establish the juvenile tests was grown at Dorena Tree Improvement Center near Cottage Grove, Oregon. Seeds, collected in 1985 and stored in freezer, were soaked in 1% H_2O_2 for 24 h, soaked in water for an additional 24 h and drained, and then stratified for 60 d at 0.6 - 1.7 °C. Visual inspections of the seeds were carried out weekly for moisture and mold. Moldy seeds were rinsed thoroughly with 1% H_2O_2.

Stratified seeds were sown in 164 ml Ray Leach Supercells (Stuewe & Sons Inc., Corvallis, OR) (2 seeds per cell) on the 17 and 18 March 1998. Sowing media consisted of peat:vermiculite:composted fir bark:perlite:pumice (40:30:10:10:10). The cells were filled to approximately 75% volume with the media and 60 g of Nutricote controlled release fertilizer (180 d release time, 18% N, 6% P and 8% K) with minor elements (1.2% Mg, 0.02% B, 0.05% Cu, 0.2% Fe, 0.06% Mn, 0.02% Mo, and 0.015% Zn) applied to each cell (0.6 g/cell). The cells then were completely filled and watered prior to sowing. Seeds were hand sown in depressions made in the surface of the cells and covered with a thin layer of grit (< 7 mm).

Each family was kept in a separate supercell rack for convenience. There were 98 cells (7 rows by 14 columns) per rack. A total of 140 cells were sown per family (20 columns, or approximately 1.5 racks per family). The racks were placed in a greenhouse after sowing and covered with screens to protect seeds from birds and rodents. The soil surface was kept moist and the greenhouse temperature was kept below 30 °C throughout the germination period. A continuously moist soil surface was maintained during the growing season until mid-July. After the primary needles emerged the seedlings were watered as needed. Dormancy was induced in late August by discontinuing regular irrigation. During the dormant season, irrigation was applied to the field capacity when soil tension reached – 0.7 bars.

In addition to fertilization at sowing, the following fertilization regime was applied during the growing season starting after emergence was completed.

Weeks 1-3 (May 4- May 22). 100 ppm Peter's Excel 15-5-15 with Ca + Mg applied once/week. One fertilization with 80 ppm Peter's Conifer Grower (20-7-19) replaced the Excel during this period.

Weeks 4-8 (May 25 – June 26). 150 ppm Peter's Excel 21-5-20 with Ca + Mg once per week. Fe supplemented 1-2 fertilizations.

Weeks 9-12 (June 29 – July 24). 100-125 ppm Peter's Conifer Grower (20-7-19) or Plantex All Purpose (20-20-20) every 10 days.

Weeks 13-19 (July 27 – Sept. 4). 150-200 ppm Peter's Excel (21-5-20) with Ca + Mg alternated with 150 ppm Peter's Conifer Grower every 7-10 days. Fe supplemented 2-3 fertilizations.

Leach heavily at week 19. Starting in mid to late September, 100 ppm Peter's Conifer Finisher (4-25-35), or similar low N, high P and K fertilizer, was applied every 7-10 days through the end of October.

After germination was completed, the seedlings in each cell were randomly thinned to leave one seedling per cell. Later, the seedlings were moved outside on raised beds where they were randomly assigned into replications. The first seedling in each column of seedlings in each rack was tagged with family information and replication number (from 1 to 20). Then the first 5 seedlings starting from the tagged seedling in each column were randomly assigned to 20 replications. Each replication was a complete block of 55 families where each family was represented by a 5-seedling row plot (three family plots in each row). A border row of seedlings was placed around each replication using the remaining seedlings. Replications were kept in this arrangement until transplanting. The integrity of these replications, at least in composition of families and row plots, was maintained throughout in remainder of the study.

3.3.2 Seedling Trial Establishment and Inoculation

On 5 January 1999, 16 of the 20 replications were put in cold storage, generously provided by The Timber Company (now part of Plum Creek Co.), near Cottage Grove, Oregon (Table 3.1). Justification for this treatment is as follows:

In natural populations of infected Douglas-fir, maximum spore release occurs in June (Michaels and Chastagner 1984). While newly emerged needles are more susceptible to infection than older needles (Stone *et al.* 1998 SNCC Annual Report), there is normally a great deal of variation in bud-burst timing in this species (Li and Adams 1993). Thus, in order to have effective inoculation with the methods mentioned in the introduction, uniform timing of budbreak among seedlings is important. Chilling of seedlings is an effective way of controlling bud-burst timing in Douglas-fir (Campbell and Sugano 1975). Thus, seedlings employed in establishing the seedling trials were chilled in cold storage at 4 °C for 114 d (until 29 April 1999).

Two seedling trials were established in coastal Oregon, one in Pleasant Valley, near Tillamook (transplanted on 14 May 1999) and the other in Toledo, near Newport (transplanted on 12 May 1999), with 4 replications at each location. The land was provided by Simpson Timber Company where surrounding Douglas-fir stands were heavily infected with *P. gaeumannii* that could provide a source of natural inoculum. The seedlings were naturally inoculated by these spores.

The remaining 8 replications were transported back to the Dorena Tree Improvement Center. When the seedlings burst bud, which occurred shortly after removal from cold storage, they were placed in a growth chamber for artificial inoculations. Infected Douglas-fir branches were collected from Sourgrass Summit (near Midway, Oregon) area one day before inoculation and were stored in a cold room overnight until inoculation. Replications were randomly placed in the chamber and a screen-top was placed over each replication. Infected branches were placed on the screen-tops with lower needle surfaces facing down on the seedlings.

Later, the screen-tops were covered with plastic sheets to the floor to keep the spores released from the branches over the seedlings. Infected branches were replaced with fresh infected branches collected from the same area 3.5 d after the start of the inoculation. Humidity was kept at 100% by misting and temperature at 20 °C at all times to provide the optimum conditions for spore release (Michaels and Chastagner 1984).

Table 3.1. Summary of how the 20 replications were used.

Replication Number	Chilled?	Inoculation method	Transplanted in...
1, 2, 3 and 4*	No	Not actively inoculated	Forestry Sciences Laboratory, Corvallis, OR
8, 10, 13 and 15	Yes	Natural	Pleasant Valley, OR.
5, 12, 14 and 19	Yes	Natural	Toledo, OR.
6, 7, 9 and 20	Yes	Suspended infected branches over seedlings in growth chamber (1 week in the chamber).	Dorena Tree Improvement Center, Cottage Grove, OR.
11, 16, 17 and 18	Yes	Same as above but were kept for another week in the chamber without infected branches.	Dorena Tree Improvement Center, Cottage Grove, OR.

* For each replication a family row position was randomly selected in each rack (same position in each family rack). Replications 1-4 were set up before the other replications and 4 randomly selected rows were designated from 1 to 4.

After one week, four randomly selected replications were removed from the chamber and the seedlings were transplanted (24 June 1999) into raised beds at Dorena. The raised beds were covered with 50% shade cloth to provide a cool moist environment for four weeks of incubation.

The remaining 4 replications were kept in the chamber for an additional week under the same conditions except the inoculum source was removed. They, too, were then transplanted on raised beds and covered with shade cloth.

The final 4 replications of the original 20, that were never put in cold storage, were used to establish a separate experiment at the U.S. Forest Service, Pacific Northwest Research Station, Forest Sciences Laboratory (FSL), Corvallis, Oregon. These seedlings were not artificially inoculated. In addition, because Corvallis is located 45 miles east of the coast natural *P. gaeumannii* spore loads were minimal, especially in comparison to the trials on the coast.

The reason for using different means of inoculation was to determine the most suitable method for early testing purposes. Seedlings were successfully infected with both inoculation methods but disease symptoms only developed in the field tests. Therefore, the data collected only from the Pleasant Valley and Toledo seedling trials are reported here.

A rectangular area about 300 m^2 at Pleasant Valley and Toledo was prepared for seedling trials. The land at Pleasant Valley had recently been transplanted with western hemlock (*Tsuga canadiensis*) and there was not any competing vegetation. At Toledo, however, the area had been clear cut and slash-burned, and salmonberry (*Rubus spectabilis*) and blackberry (*Rubus fruticosa*) claimed the area. While no soil preparation was necessary, except to remove already planted hemlock trees, at Pleasant Valley, soil at Toledo was tilted prior to transplanting.

In the following years, the competing vegetation was removed by hand in Pleasant Valley (primarily grass) and by herbicide in Toledo (primarily *Rubus* sp.). Remnants of thick competing vegetation at Toledo blocked air circulation around

trees and created ever-present wet conditions and thus predisposing the trees to a common nursery disease, gray mould, caused by *Botrytis cinerea*. A total of 631 trees were affected and 58 trees were killed by gray mould (total number of dead trees = 131) in Toledo. Only 17 trees were lost in Pleasant Valley.

The statistical design was a randomized complete block, with four replications at each site where each family was represented by a five-tree row plot in each replication. Spacing was 60 cm (at Pleasant valley) or 45 cm (at Toledo) between rows and 30 cm 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.

3.3.3 Field Progeny Tests

The mature progeny test plantations are also located in Coastal Oregon (Figure 3.1, Table 3.2), in the Siuslaw National Forest. These progeny tests were established as a part of USFS breeding program in 1986 using 1-0 container grown seedlings. Survival of the trees was good at the time of the assessments (86.6% in Gordy, and 67.3% in Salal). Trees in these plantations were naturally infected with *P. gaeumannii*.

The statistical design is a sets-in-replications design with five replications of four-tree non-contiguous family plots in each replication. One hundred and five families were divided into 3 sets of 35 families each. At each site, families were blocked by set (sub-blocks) with sets randomly assigned to sub-blocks. Within each of 5 replicated blocks, families were represented by a 4-tree non-contiguous plot in each sub-block. The 55 families used in this study were selected from two of the three sets (28 families from set number 2, and 27 families from set number

3). Tree spacing was 3 m by 3 m at planting. The tests were fenced to exclude browsing.

Table 3.2. Information on seedling trials and progeny tests in this study.

	Site			
	Juvenile		Mature	
	Pleasant Valley	Toledo	Salal	Gordy
Elevation (m)	62	110	100	265
Latitude	45° 21' N	44° 37' N	45° 08' N	44° 55' N
Longitude	123° 48' W	123° 57' W	123° 53' W	123° 57' W
Survival[a)] (%)	99	94	67	87
Year planted	1999	1999	1986	1986
Ownership	Simpson Timber Co.	Simpson Timber Co.	USFS	USFS

[a)] Survival of seedlings in the assessed families one year after planting at the juvenile sites and in 1996 for the mature sites.

3.3.4 Traits and Assessment Methods

Trees in the seedling trials and mature progeny tests were assessed for foliage traits in the field (Table 3.3). Additional assessments were conducted in the laboratory on the needle samples collected from the seedling trials.

Between 29 May and 9 June 2000, two-year-old seedlings were visually assessed for needle color, needle retention, foliage density and foliage color at Pleasant Valley and Toledo (Table 3.3). After the field assessments were completed, needle samples were collected for further assessments in the laboratory in an attempt to find additional, more precisely measured traits to be employed in early testing. The collection procedure was as follows; first, the first branch that contained needles produced in 1999 from the top and on the south side of each tree was identified. Second, the internode with the 1999 needle complement was

Table 3.3. Descriptions of traits and measurement methods.

Trait	Description and measurement method
Juvenile (field)	Assessed both in Summer 2000 and Fall 2000.
Needle color	Visually scored on a south facing single internode on a scale from 1 (yellow) to 3 (green)
Needle retention	Visually scored on the same internode as above on a scale from 0 (0-10% retention) to 9 (91-100% retention).
Foliage density	Visually scored over the entire seedling on a scale from 1 (sparse) to 6 (dense).
Foliage color	Visually scored over the entire seedling on a scale from 1 (yellow) to 3 (green).
Juvenile (lab.)	Assessed on a sample internode collected from the south side of each seedling in Summer 2000.
Needle color	Visually scored on the sample internode by comparing it to a color photograph of 4 different needle colors (1 = yellow to 4 = green) (see Figure 3.3).
Retention	Visually assessed from 0 (0 - 10% retention) to 9 (91 – 100% retention).
Dry weight* (g)	Dry weight of selected 50 needles.
Needle length (mm)	Length of 10 randomly selected needles (included in the 50 above).
Stomata with pseudothecia (%)	Percent stomata clogged with pseudothecia, assessed on the central 50 stomata of the above 10 needles in the second stomata row at the right side of needle midrib needle petiole facing observer.
Needle specific area* (cm^2/g)	Projected needle specific area calculated as needle weight/projected needle area.
Fungal DNA* (pg)	Amount of fungal DNA in needle samples determined by quantitative PCR.
Mature	
Foliage density	Visually scored over entire crown from 1 (sparse) to 6 (dense).
Foliage color	Visually scored over entire crown from 1 (yellow) to 3 (green).
Needle retention	Visually scored on an internode with 1-year-old needles from 0 (0 - 10% retention) to 9 (91 - 100% retention).

*)Single value per family plot per replication.

clipped and taped at the base with one of 5 different colors of tape to identify each tree's position in its family row plot. Then all 5 taped internodes from each family plot were placed in a plastic bag along with an index card bearing site, replication and family information. The same information was also placed on the bags themselves with permanent markers. Bagged samples were immediately placed in on ice and transported at the end of the day to FSL. Finally, the samples were stored in a freezer at –10 °C until the assessments were conducted.

The following steps were followed during the laboratory assessments. First, a bag containing samples was pulled from the freezer. For each family plot, two random needles were removed from each sample internode (10 needles total) and placed in a screw cap micro-centrifuge tube (Island Scientific, IS-522) along with two solid glass beads (Fisher Scientific, 11-312C) for DNA extractions. Tubes were marked with an identification number that corresponded to their site, replication and family information. The tubes containing needles were kept in the freezer until extraction. Later, after thawing, the branch samples were lined on black cardboard according to their tape color. Needle color was first assessed visually comparing the sample color with a color photo of needles exhibiting 4 distinct color groups (Figure 3.2).

Next, needle retention was estimated. To determine the estimation method, needle retention was first visually scored on 50 samples from 10 families on a scale from 0 (0 - 10% retention) to 9 (91 - 100% retention) and then actual numbers of needles and needle scars on each sample were counted and retention was calculated by dividing the number of needles by the total number of needle scars and needles. Although the latter method would precisely provide percent needle retention, it was very time consuming. A simple correlation analysis revealed that the estimates obtained from the two methods were highly correlated ($r = 0.97$, $P < 0.001$). Thus, needle retention was subsequently visually estimated in the laboratory as it was in the field.

Figure 3.2. Color photograph of four needle color groups used in the laboratory assessments of needle color.

From each individual internode sample, 10 needles were randomly selected per tree (50 needles per family plot). These needles were bulked for each family plot and weighed, while keeping two needles from each individual tree identified. After weighing, the identified needles (10 needles total) were placed on a 10 cm by 20 cm clear glass plate (lower side of needles facing up) side by side, with petioles facing in the same direction. The remaining 40 needles were also placed on the same glass plate and were spaced so that they did not touch each other. Then, the

needles were fixed into these positions by putting another glass plate on them and then binding the two plates with paper clips (Figure 3.3).

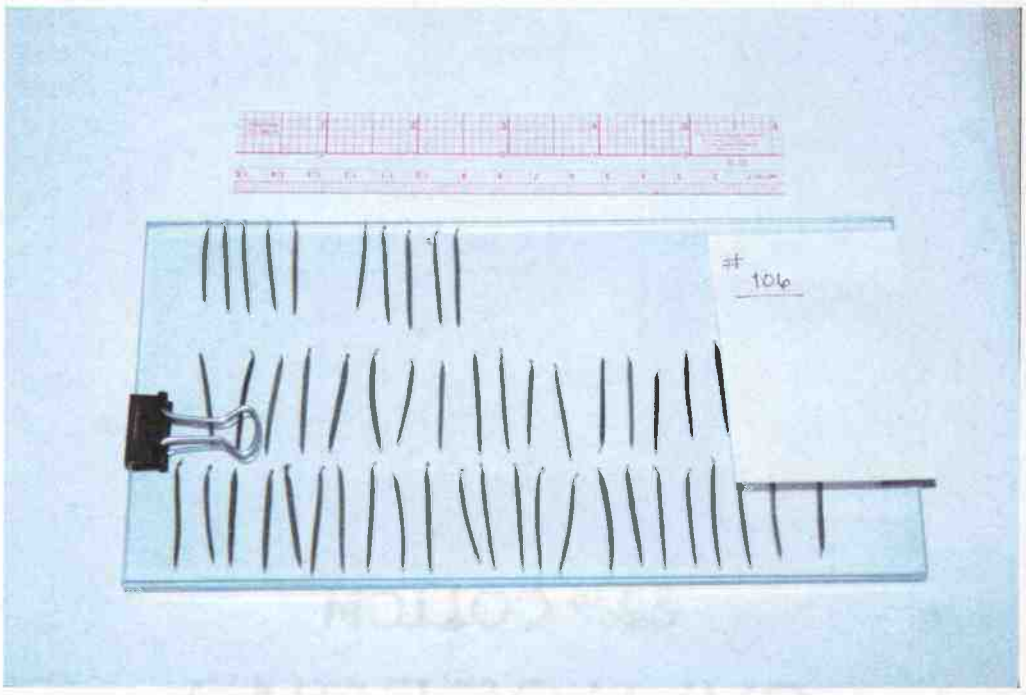

Figure 3.3. Glass plates with 50 needles from a single family plot between them. Ten needles in upper left corner come from each of 5 trees (single internode, 2 needles from each seedling) in the plot. Individual tree identities of these needles were maintained. For the remaining 40 needles (8 needles from each seedling), individual tree identities were not maintained.

The midpoints of each of the 10 identified needles were marked with a pen. Then 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 for per family plot) for presence or absence of fungal pseudothecia (Figure 3.4) to estimate proportion of stomata occluded with the fungal fruiting body. The lengths of these 10 needles were also measured.

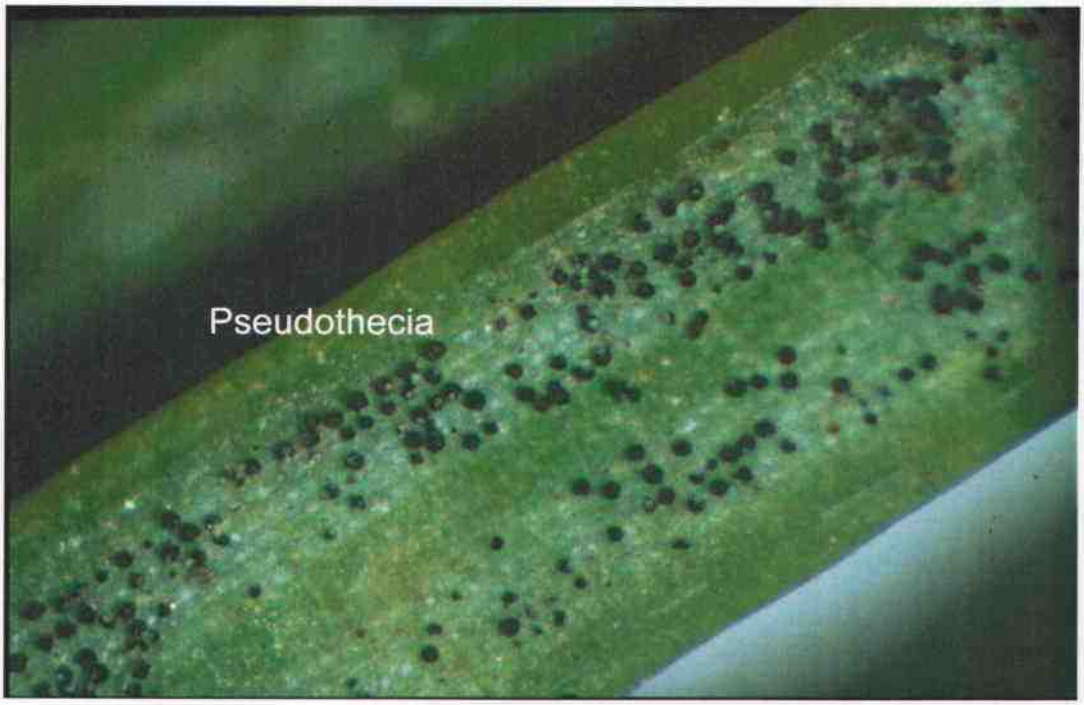

Figure 3.4. Under surface of a Douglas-fir needle infected by *P. gaeumannii* with its pseudothecia emerged from stomata openings.

Projected area of the 50 needles was measured using a video image recorder and AgVision software (Decagon Devices, Inc., Pullman, WA) software in Barbara Bond's laboratory (Department of Forest Science, Oregon State University, Corvallis, OR) and needles placed in small envelopes for drying. Finally, 24-hour air-dried needles were weighed.

Total genomic DNA was extracted from the 10 needles placed in the micro-centrifuge tubes. The needles were frozen in liquid nitrogen and pulverized in a Mini-Beadbeater (Biospec Products, Bartlesville, OK) for 30 s at 4200 rpm. After pulverization, samples were incubated in 1.5 ml extraction buffer (2% CTAB (cetyltrimethylammonium bromide), 100 mM Tris, pH 8.0, 20 mM Na_2EDTA pH 8.0, 1.4 M NaCl, 1% polyvinylpolypyrrolidone, 0.1 % 2-mercaptoethanol) at 65 °C for 2 hours. The DNA was purified in 24:1 chloroform:isoamyl alcohol,

precipitated from the aqueous phase by the addition of isopropanol, washed in 70% ethanol, and resuspended in 1 ml TE (5 mM Tris, pH 8.0, 0.5 mM Na_2EDTA).

Real-time PCR was employed to quantify *P. gaeumannii* DNA (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) chemistry (Livak *et al.* 1995, Gibson *et al.* 1996, and Heid *et al.* 1996) in conjunction with the 7700 Sequence Detection System (PE Applied Biosystems). The fleurogenic Taqman probe, labeled 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'
of Taq DNA polymerase cleaves annealed probe molecules. Release of the reporter dye results in an intense fleurescent signal which is measured by the 7700 Sequence Detection System during each cycle of the PCR process.

Reactions were performed in 15 µl aliquots with 1X TaqMan Universal Master Mix (PE Applied Biosystems), 150 nM *P. gaeumannii* FAM-labeled probe, 60 nM forward and reverse primers for *P. gaeumannii*, and 5 µl DNA template. Real-time quantitative PCR was performed by an automated ABI Prism 7700 SDS in MicroAmp optical 96-well plates or single tubes (PE Applied Biosystems). Thermal cycling was completed in less than 2 h and conditions consisted of 10 min at 50 °C and 5 min at 95 °C and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The 7700 SDS software collected data for reporter dye every 7 s from each well, generating a fluorescence data for each amplification. The threshold cycle (CT) was recorded for dye as the cycle at which fluorescent signal, associated with and exponential growth of PCR product, exceeded background fluorescence. Every assay included genomic DNA standard for *P. gaeumannii.* The standard was run in quadruplicate for each assay. A standard curve for *P. gaeumannii* was generated by plotting known DNA amounts against CT-values calculated by the SDS software and calculating a regression equation. Unknown samples were quantified from measured CT-values by interpolation using the regression equation.

Each sample was subjected to PCR twice. Due to large sample size PCR was conducted in several batches. In order to remove batch effects, results for each sample was 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.

Field assessments for the Toledo and Pleasant Valley seedling trials were repeated in September 2000, after bud set.

While some traits are directly related to SNC symptoms (i.e., needle color, needle retention, foliage density, and foliage color) and signs (i.e., proportion of stomata occluded with pseudothecia and amount of fungal DNA), others are not directly related to SNC, but to needle size (i.e., needle dry weight, needle length and needle specific area). The latter traits were measured because of the possibility that they might be good predictors of SNC tolerance in older trees. If this would be the case, objectively measured needle size traits would replace subjectively scored SNC symptoms.

Field assessments of the trees at the mature progeny tests sites were conducted in the spring of 1996 and 1998 (at ages 10 and 12) (Table 3.3). While foliage density and color ratings were based on the entire tree crown, needle retention was scored on branch internodes produced in the previous growing season.

3.3.5 Data Analysis and Genetic Calculations

Data analyses were conducted using the SAS statistical package (SAS Institute 1990). The UNIVARIATE and MEANS procedures were used to calculate overall means, ranges among family means, standard deviations and coefficients of variation. All 18 traits (Table 3.3) were subjected to analysis of variance (ANOVA), first for each individual test and then with the data sets

combined over sites in each age group. Due to differences in statistical design in seedling trials versus mature progeny tests different random models were employed.

The linear model for individual seedling trials was:

$$y_{jkl} = \mu + r_j + f_k + rf_{jk} + e_{jkl}$$

where, y_{jkl} is the observation on the l_{th} tree of the k_{th} family in the j_{th} replication; μ is the overall mean; r_j is the effect of the j_{th} replication, E(r_j)=0, Var(r_j)=σ_r^2; f_k is the effect of the k_{th} family, E(f_k)=0, Var(f_k)=σ_f^2; rf_{jk} is the interaction between the k_{th} family and the j_{th} replication, E(rf_{jk})=0, Var(rf_{jk})=σ_{rf}^2; and e_{jkl} is the random error, E(e_{jkl})=0, Var(e_{jkl})=σ_e^2.

The linear model for combined data over seedling trials was:

$$y_{ijkl} = \mu + s_i + r_{j(i)} + f_k + sf_{ik} + rf(s)_{jk(i)} + e_{ijkl}$$

where, y_{ijkl} is the observation on the l_{th} tree of the k_{th} family in the j_{th} replication in the i_{th} site; μ is the overall mean; s_i is the effect of the i_{th} site, E(s_i)=0, Var (s_i)=σ_s^2; $r_{j(i)}$ is the effect of the j_{th} rep at the i_{th} site, E($r_{j(i)}$)=0, Var($r_{j(i)}$)=σ_r^2; f_k is the effect of the k_{th} family, E(f_k)=0, Var(f_k)=σ_f^2; sf_{ik} is the interaction between the i_{th} site and the k_{th} family, E(sf_{ij})=0, Var(sf_{ij})=σ_{sf}^2; $rf(s)_{jk(i)}$ is the interaction between the k_{th} family and the j_{th} rep in the i_{th} site, E($rf(s)_{jk(i)}$)=0, Var($rf(s)_{jk(i)}$)=$\sigma_{rf(s)}^2$; and e_{ijkl} is the random error, E(e_{ijkl})=0, Var(e_{ijkl})=σ_e^2.

For traits with a single value per family plot in each replication (i.e., dry weight, needle specific area, and fungal DNA, Table 3.3), statistical models were the same as above but without random error term:

$$y_{jk} = \mu + r_j + f_k + rf_{ik}$$

for individual sites, and

$$y_{ijk} = \mu + s_i + r_{j(i)} + f_k + sf_{ik} + rf(s)_{jk(i)}$$

for the data combined over the sites.

The ANOVA was conducted for data from individual mature sites according to the following linear model:

$$y_{jklm} = \mu + r_j + d_k + rd_{jk} + f(d)_{l(k)} + rf(d)_{jl(k)} + e_{jklm},$$

where, y_{jklm} is the observation on the m_{th} tree of the l_{th} family in the j_{th} replication and k_{th} set; μ is the overall mean; r_j is the effect of the j_{th} replication, $E(r_j) = 0$, $Var(r_j) = \sigma_r^2$; d_k is the effect of the k_{th} set, $E(d_k) = 0$, $Var(d_k) = \sigma_d^2$; rd_{jk} is the interaction effect between j_{th} replication and k_{th} set, $E(rd_{jk}) = 0$, $Var(rd_{jk}) = \sigma_{rd}^2$; $f(d)_{l(k)}$ is the effect of the l_{th} family in the k_{th} set, $E(f(d)_{l(k)}) = 0$, $Var(f(d)_{l(k)}) = \sigma_{f(d)}^2$; $rf(d)_{jl(k)}$ is the interaction effect between the j^{th} replication and the l^{th} family in the k^{th} set, $E(rf(d)_{jl(k)}) = 0$, $Var(rf(d)_{jl(k)}) = \sigma_{rf(d)}^2$; e_{jklm} is the random error $E(e_{jklm}) = 0$, $Var(e_{jklm}) = \sigma_e^2$.

The model for the data set combined over mature sites was:

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

where, y_{ijklm} is the observation on the m_{th} tree of the l_{th} family in the j_{th} replication and k_{th} set in the i_{th} site; μ is the overall mean; s_i is the effect of the i_{th} site, $E(s_i)=0$, $Var(s_i)=\sigma_s^2$; $r(s)_{j(i)}$ is the effect of the j_{th} replication in the i_{th} site, $E(r(s)_{j(i)}) = 0$, $Var(r(s)_{j(i)}) = \sigma_{r(s)}^2$; d_k is the effect of the k_{th} set, $E(d_k) = 0$, $Var(d_k) = \sigma_d^2$; sd_{ik} is the effect of interaction between the i_{th} site and k_{th} set, $E(sd_{ik}) = 0$, $Var(sd_{ik}) = \sigma_{sd}^2$; $rd(s)_{jk(i)}$ is the effect of interaction between the j_{th} replication and k_{th} set in the i_{th} site, $E(rd(s)_{jk(i)}) = 0$, $Var(rd(s)_{jk(i)}) = \sigma_{rd(s)}^2$; $f(d)_{l(k)}$ is the effect of the l_{th} family in the k_{th} set, $E(f(d)_{l(k)}) = 0$, $Var(f(d)_{l(k)}) =$

$\sigma^2_{f(d)}$; $sf(d)_{il(k)}$ is the effect of interaction between the i_{th} site and the l_{th} family in the k_{th} set, $E(sf(d)_{il(k)}) = 0$, $Var(sf(d)_{il(k)}) = \sigma^2_{sf(d)}$; $srf(d)_{ilj(k)}$ is the effect of interaction between the i_{th} site, the l_{th} family and the k_{th} set in the j_{th} replication, $E(srf(d)_{ilj(k)}) = 0$, $Var(srf(d)_{ilj(k)}) = \sigma^2_{srf(d)}$; e_{ijklm} is the random error, $E(e_{ijklm}) = 0$, $var(e_{ijklm}) = \sigma^2_e$.

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

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 (Appendix B).

Analyses of variance were conducted using PROC GLM (generalized linear models) of the SAS statistical package to test significance of family differences (Type III sums of squares). Forms of the expected mean squares are given in Table 3.4. 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).

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 correlations among traits. Because wind-pollinated families in this study came from parent trees in wild stands, additive genetic variance (σ^2_A) was estimated as four times the family component of variance, and assumes individuals in these families are true half-sibs. For individual sites, total phenotypic variance (σ^2_{PI}) was estimated as (see Table 3.4 for description of terms),

$$\sigma^2_{PI_J} = \sigma^2_e + \sigma^2_{rf} + \sigma^2_f, \text{ for juvenile traits,}$$

$$\sigma^2_{PI_M} = \sigma^2_e + \sigma^2_{rf(d)} + \sigma^2_{f(d)}, \text{ for mature traits,}$$

and total phenotypic variation among family means (σ^2_{PF}) as,

$$\sigma^2_{PF_J} = \frac{\sigma^2_e}{rt} + \frac{\sigma^2_{rf}}{r} + \sigma^2_f, \text{ for juvenile traits,}$$

$$\sigma^2_{PF_M} = \frac{\sigma^2_e}{rt} + \frac{\sigma^2_{rf(d)}}{r} + \sigma^2_{f(d)}, \text{ for mature traits.}$$

For the data combined over the sites, total phenotypic variance (σ^2_{PI}) was estimated as (see table 3.4 for description of terms),

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

$$\sigma^2_{PI_M} = \sigma^2_e + \sigma^2_{srf(d)} + \sigma^2_{sf(d)} + \sigma^2_{f(d)}, \text{ for mature traits,}$$

and total phenotypic variation among family means (σ^2_{PF}) as,

$$\sigma^2_{PF_J} = \frac{\sigma^2_e}{srt} + \frac{\sigma^2_{rf(s)}}{sr} + \frac{\sigma^2_{sf}}{s} + \sigma^2_f, \text{ for juvenile traits,}$$

$$\sigma^2_{PF_M} = \frac{\sigma^2_e}{srt} + \frac{\sigma^2_{srf(d)}}{sr} + \frac{\sigma^2_{sf(d)}}{s} + \sigma^2_{f(d)}, \text{ for mature traits.}$$

Due to unbalance in the data, r (mean number of replications) and t (mean number of trees per plot) used in above analyses were estimated by the GLM procedure. Narrow-sense individual (h^2_i) and family (h^2_f) heritabilites were estimated following Falconer (1981).

$$h^2_i = \frac{\sigma^2_A}{\sigma^2_{PI}} = \frac{4\sigma^2_f}{\sigma^2_{PI}} \text{ and } h^2_f = \frac{\frac{1}{4}\sigma^2_A}{\sigma^2_{PF}} = \frac{\sigma^2_f}{\sigma^2_{PF}},$$

using the respective variance component estimates for juvenile and mature traits. Standard errors of heritability estimates were calculated following Dickerson (1969). In the estimates for mature tests σ^2_f is replaced with $\sigma^2_{f(d)}$. Heritability estimates based on single sites are biased because any genotype-by-environment

interaction is confounded in the estimate of the family variance component (Comstock and Moll 1963).

Data were standardized for each trait to remove scale effects by subtracting the site mean and dividing by the site standard deviation prior to analyses for the genetic correlation calculations. Additive genetic correlations between traits within each age group were estimated as

$$r_A = \frac{Cov_f(x,y)}{\sqrt{\sigma^2_{fx} \cdot \sigma^2_{fy}}},$$

where, r_A is the additive genetic correlation between traits x and y, $Cov_f(x,y)$ is the family covariance between traits x and y, estimated as

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

Table 3.4. Form of ANOVA and expected mean squares for seedling trials and mature progeny tests[a)].

Source	Degrees of freedom	Expected Mean Squares
Juvenile*		
Site	s-1	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tr\sigma_{sf}^2 + tf\sigma_{r(s)}^2 + trf\sigma_s^2$
Replication(Site)	(r-1)s	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tf\sigma_{r(s)}^2$
Family	f-1	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tr\sigma_{sf}^2 + trs\sigma_f^2$
Site*Family	(s-1)(f-1)	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tr\sigma_{sf}^2$
Rep*Family(Site)	(r-1)(f-1)s	$\sigma_e^2 + t\sigma_{rf(s)}^2$
Plot Error	(t-1)rfs	σ_e^2
Mature		
Site	s-1	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tr\sigma_{sf(d)}^2 + tf\sigma_{rd(s)}^2 + trf\sigma_{sd}^2 + tdf\sigma_{r(s)}^2 + trdf\sigma_s^2$
Replication(Site)	(r-1)s	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tf\sigma_{rd(s)}^2 + tdf\sigma_{r(s)}^2$
Set	d-1	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tr\sigma_{sf(d)}^2 + trs\sigma_{f(d)}^2 + tf\sigma_{rd(s)}^2 + trf\sigma_{sd}^2 + trsf\sigma_d^2$
Site*Set	(s-1)(d-1)	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tr\sigma_{sf(d)}^2 + tf\sigma_{rd(s)}^2 + trf\sigma_{sd}^2$
Set*Replication(Site)	(d-1)(r-1)s	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tf\sigma_{rd(s)}^2$
Family(Set)	f-d	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tr\sigma_{sf(d)}^2 + trs\sigma_{f(d)}^2$

Table 3.4 (Continued)

Site*Family(Set)	(s-1)(f-d)	$\sigma_e^2 + t\sigma_{srf(d)}^2 + tr\sigma_{sf(d)}^2$
Site*Replication*Family(Set)	(s-1)(f-d)r	$\sigma_e^2 + t\sigma_{srf(d)}^2$
Plot Error	(t-1)rfs	σ_e^2

* For variables with single value per family plot per replication, Plot Error drops out and Rep*Family(Site) becomes the overall error term in the model.

[a)] Assumes balanced design. s = number of sites, t = number of trees family per plot, r = number of replications, f = number of families, d = number of sets, $\sigma_{rf(s)}^2$ = variance component for replication-by-family interaction within site, σ_{sf}^2 = variance component for site-by-family interaction, $\sigma_{r(s)}^2$ = variance component for replication within site, σ_s^2 = variance component for site, σ_f^2 = variance component for family, $\sigma_{srf(d)}^2$ = variance component for site-by-replication-by-family interaction within sets, $\sigma_{sf(d)}^2$ = variance component for site-by-family interaction within set, $\sigma_{rd(s)}^2$ = replication-by-set interaction within sites, σ_{sd}^2 = variance component for site-by-set interaction, $\sigma_{f(d)}^2$ = variance component for families within sets, σ_d^2 = variance component for set, and σ_e^2 = the plot error.

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

$$r_B = \frac{r_{xy}}{\sqrt{h_{fx}^2 \cdot h_{fy}^2}},$$

where, r_B is the genetic correlation between the juvenile (x) and the mature (y) traits; r_{xy} is the family mean correlation between the juvenile (x) and the mature (y) traits; h_{fx}^2 and h_{fy}^2 are the respective estimates of family mean heritabilities of the juvenile and mature traits.

In order to determine efficiency of early selection for Swiss needle cast resistance in Douglas-fir, expected gains from direct selection, correlated response from indirect selection of correlated traits at the same or 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):

$$G_y = 2 \cdot i \cdot h_f^2 \sqrt{\sigma_{PF}^2},$$

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

$$CR_y = 2 \cdot i \cdot r_A \sqrt{h_{fy}^2 \cdot h_{fx}^2 \cdot \sigma_{PF(y)}^2},$$

where, r_A is the genetic correlation between traits x and y, h_{fy}^2 and h_{fx}^2 are the respective family heritabilities, and $\sigma_{PF(y)}^2$ is the phenotypic variance of family means for trait y. Efficiency of early selection (RE) was estimated as the ratio of the correlated response (CR_m) in a trait at mature age (m) when selection was

applied to a comparable (or different) trait at juvenile age (j) to response expected from direct selection at the mature age (G_m):

$$RE = \frac{CR_m}{G_m} = \frac{2 \cdot i \cdot r_A \sqrt{h^2_{f(m)} \cdot h^2_{f(j)} \cdot \sigma^2_{PF(m)}}}{2 \cdot i \cdot h^2_f \sqrt{\sigma^2_{PF(m)}}} = r_A \sqrt{\frac{h^2_{f(j)}}{h^2_{f(m)}}}.$$

The primary means of infection of Douglas-fir needles by *P. gaeumannii* is airborne spores, starting in the spring and continuing throughout the summer. Secondary infections occur starting in winter when hyphae growing on the needle surface penetrate stomata openings (Capitano 1999). As in many experiments involving airborne pathogens, heterogeneous distribution of inoculum across the plots can affect the precision and reliability of results and estimates (Jenkyn *et al.* 1996). Various statistical procedures have been proposed to take account of such variation (Bartlett 1978, Wilkinson *et al.* 1983, Papadakis 1984). Although amount of inoculum was not quantified for any of tests (juvenile or mature), 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 with the air. Second, in *P. gaeumannii,* spore release is not a one-time occurrence, but a continuous process throughout spring and summer. Thus, plot-to-plot variation in inoculum density was probably negligible.

Variograms for each replication at each site were produced using plot means for fungal DNA in seedling trials and individual tree values for needle retention in mature tests. A variogram is a plot that indicates if there are spatial patterns in data. First, squared differences between data pairs for individuals closest to each other are averaged over the total number of pairs for that specific distance. This procedure is repeated for each increasing distance between pairs. Finally, the variogram is produced using the averages for each distance class. If plots that are closer to each other are more similar to each other than further spaced plots, an increasing trend is expected in the variogram. The amount of fungal DNA

was chosen because it would be the best indicator of any differences in inoculum density. No spatial pattern was observed for fungal DNA content of needles (Figure 3.5). Variograms of needle retention from mature tests were similar to what was observed for fungal DNA from the seedling trials. This indicates that trees close to each other are as different from each other as trees spaced larger distances apart.

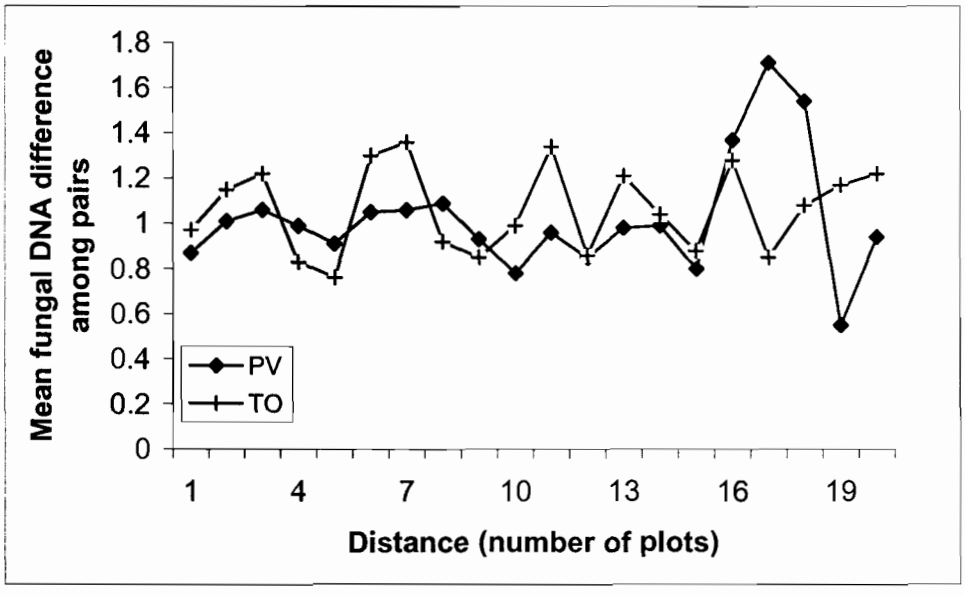

Figure 3.5. Variogram of fungal DNA content for one replication of each seedling trial at Pleasant Valley (PV) and Toledo (TO). (See Appendix B for variograms of other replications.).

3.4 Results

On average, the severity of SNC disease symptoms and variation among family means for these traits were greater in the Pleasant Valley seedling trial than at Toledo (Table 3.5). The exception was that the proportion of stomata occluded with pseudothecia was greater at Toledo (Table 3.5). Although the mature tests at

Salal and Gordy had similar symptom severity, family variation was higher in Salal (Table 3.5).

Preliminary investigations indicated that for three of the juvenile traits assessed in the laboratory (needle color, proportion of stomata occluded with pseudothecia and projected needle specific area) there were no statistically significant differences among families in either trial. In addition, family means for foliage density, foliage color, dry weight and needle length were only statistically significantly different at Pleasant Valley. When the data from both seedling trials were combined, however, significant differences among family means for all traits were observed (Table 3.7, Appendix B) except for fungal DNA content. Thus, fungal DNA was excluded from the analyses. Analyses were conducted on this combined data set because there was no significant family-by-site interaction (except for barely significant family-by-site interaction for field needle color in summer, $P = 0.0438$, Appendix B) among the traits to warrant to keep the sites separate.

Among the juvenile traits assessed in the field in fall, only needle retention and foliage density significantly differed among families (Appendix B). Genetic correlations between the same traits assessed in summer and fall were moderate but significant (0.57 for foliage density, 0.68 for needle retention). In addition there were no significant relationships between juvenile traits assessed in the fall and mature traits. Therefore, a single assessment in the summer is sufficient and the traits assessed in fall will no longer be discussed.

For the mature tests, family differences were significant for all traits at both sites. Analyses were carried out also using data combined over Salal and Gordy, where average of age 10 and 12 assessments were used (Appendix B).

For the remainder of this chapter, all reported results were obtained from combined data sets over sites.

Table 3.5. Overall means, family mean ranges and standard deviations of family means for the traits at each site.

	Juvenile				Mature			
	Pleasant Valley		Toledo		Salal		Gordy	
Traits	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Needle color	1.77 (1.20-2.40)	0.25	2.01 (1.50-2.45)	0.23				
Needle retention	7.40 (3.30-8.75)	0.91	8.33 (6.75-9.00)	0.53	6.61 (4.96-8.15)	0.72	7.65 (6.47-8.36)	0.40
Foliage density	4.41 (2.30-5.65)	0.62	4.92 (3.60-5.70)	0.45	3.83 (3.04-4.77)	0.35	3.81 (3.37-4.34)	0.23
Foliage color	1.84 (1.15-2.30)	0.23	2.12 (1.63-2.47)	0.19	2.03 (1.54-2.65)	0.21	1.93 (1.67-2.25)	0.12
Laboratory								
Needle color	2.61 (2.00-3.15)	0.21	2.75 (2.32-3.25)	0.19				
Needle retention	7.17 (4.44-8.05)	0.63	7.53 (6.45-8.47)	0.45				
Dry weight	0.13 (0.11-0.19)	0.02	0.15 (0.10-0.19)	0.02				
Needle length	18.76 (15.23-23.11)	1.55	20.14 (17.02-23.33)	1.59				
Stomata with Pseudohecia	30.72 (19.69-41.20)	5.48	34.80 (17.55-56.83)	8.09				
Specific area	36.82 (31.95-46.35)	2.55	34.56 (28.66-44.44)	2.76				

Tree-to-tree (within-plot) variance accounted for the bulk of the variance (76 to 91%) for all traits under investigation (Table 3.6).

All traits under investigation were under low to moderate genetic control (Table 3.7). Heritability estimates were higher in Pleasant valley than in Toledo. When the data were combined over the two sites, individual tree heritability estimates were between the values obtained from each individual site, and family heritability estimates were equal or higher than the estimates from the individual sites, except for needle color, where a significant site-by-family interaction exists.

Heritability estimates for needle color assessed in the laboratory were lower than for needle retention assessed in the laboratory, but these estimates were similar to those for the same traits assessed in the field (Table 3.7).

Unlike fungal DNA content, the second disease sign assessed in this study, proportion of stomata occluded with pseudothecia, was significantly different among families when data were combined over seedling tests. The individual and family heritability estimates of this trait however, were low (Table 3.7). Family heritability estimates for needle morphology traits were similar in magnitude with the estimates for proportion of stomata occluded with pseudothecia.

Heritability estimates were higher for needle retention and foliage density and lower for foliage color in Gordy than in Salal for the mature traits (Table 3.7). There was significant site-by-family interaction for needle retention and heritability estimates were lower for this trait in combined data set over sites than that of the individual sites. Heritability estimates for foliage density were higher for the combined data set than for the individual tests.

Individual narrow-sense heritability estimates are in agreement with previous 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).

Table 3.6. Estimated test means (range over families in parentheses), variance components (expressed as interclass correlation coefficients), and total phenotypic variances for thirteen traits.

		Variance Components (%)				
Trait	Means	Family	Site*Family	Plot	Within Plot	Phenotypic Variance
Juvenile (field)						
Needle color	1.89 (1.54-2.26)	3.63	2.00[a]	3.40	90.97	0.4997
Needle retention	7.85 (5.20-8.80)	5.43	1.93	10.36	82.28	3.9190
Foliage density	4.66 (3.30-5.68)	6.12	0.30	10.17	83.41	2.2204
Foliage color	1.98 (1.53-2.32)	3.78	0.71	9.56	85.95	0.3883
Juvenile (lab.)						
Needle color	2.68 (2.37-3.00)	1.55	0	16.63	81.82	0.4584
Retention	7.35 (5.50-8.14)	5.23	2.02	7.80	84.95	2.1516
Dry weight* (g)	0.14 (0.10-0.18)	7.60	23.03	69.37	--	0.0013
Needle length (mm)	19.42 (16.62-22.20)	3.88	0.48	19.48	76.16	17.9599
Stomata with pseudothecia (%)	32.67 (22.05-45.84)	3.48	0	18.98	77.54	393.8942

Table 3.6 (Continued)

		Variance Components (%)				
Trait	Means	Family(Set)	Site*Family(Set)	Plot	Within Plot	Phenotypic variance
Needle specific area* (cm^2/g)	35.64 (30.57-41.14)	8.47	18.73	72.80	--	22.51
Mature						
Foliage density	3.81 (3.39-4.47)	8.94	0.36	4.92	85.78	0.5190
Foliage color	1.97 (1.61-2.42)	5.12	4.46	4.62	85.80	0.1690
Needle retention	7.20 (6.30-8.26)	2.85	6.71[a]	4.51	85.94	1.8909

[a] Significant site-by-family interaction.
* Single value per family plot per replication.

Table 3.7. Individual (I) and family mean (F) heritability estimates for Swiss needle cast traits at juvenile and mature ages.

	Juvenile (Age 2)						Mature (Averaged over ages 10 and 12)					
	Pleasant V.		Toledo		Combined		Gordy		Salal		Combined	
Field traits	I	F	I	F	I	F	I	F	I	F	I	F
Needle color	0.24	0.53	0.12	0.46	0.11	0.49*	NA	NA	NA	NA	NA	NA
Needle retention	0.37	0.56	0.12	0.39	0.20	0.60	0.43	0.64	0.33	0.51	0.13	0.33*
Foliage density	0.39	0.60	NS	NS	0.20	0.64	0.36	0.62	0.36	0.51	0.37	0.73
Foliage color	0.26	0.50	NS	NS	0.14	0.50	0.19	0.43	0.49	0.61	0.21	0.52
Laboratory traits												
Needle color	NS	NS	NS	NS	0.06	0.26						
Needle retention	0.36	0.59	0.19	0.40	0.21	0.57						
Dry weight	NA	0.45	NS	NS	NA	0.40						
Needle length	0.25	0.42	NS	NS	0.16	0.45						
Stomata with pseudothecia	NS	NS	NS	NS	0.14	0.43						
Needle specific area	NA	NS	NS	NS	NA	0.41						

* Significant family-by-site interaction. NA: Not available. NS: Non-significant family differences.

Family rankings were roughly consistent from site to site as indicated by the moderate but significant genetic correlations between sites, except for needle retention assessed at the mature sites and for juvenile needle dry weight (assessed in the laboratory) (Table 3.8).

Table 3.8. Family mean and genetic correlations of Swiss needle cast traits between juvenile (Pleasant Valley and Toledo) and mature (Salal and Gordy) sites.

Trait	Juvenile		Mature	
Field	Family	Genetic	Family	Genetic
Needle color	0.33*	0.66	NA	NA
Needle retention	0.44*	0.93	0.25	0.44
Foliage density	0.47*	1.21	0.60*	1.07
Foliage color	0.34*	0.99	0.35*	0.69
Laboratory				
Needle color	0.28*	NA		
Needle retention	0.39*	0.80		
Dry weight	0.24	0.69		
Needle length	0.30*	0.98		
Stomata with pseudothecia	0.37*	2.58		
Needle specific area	0.29*	1.15		

Estimated genetic correlations among juvenile foliage traits assessed in the were high (Table 3.9), suggesting that these traits are controlled largely by the same sets of genes. Genetic correlations between the field and the laboratory assessments of needle color and needle retention were unity (Table 3.9). Since both assessment methods yielded the same results, the less-time-consuming and less labor-intensive field assessments of these traits should be preferred over laboratory assessments.

While correlation (phenotypic and genetic estimates) among foliage color, foliage density, and needle retention were moderately strong and positive, they were somewhat weaker in magnitude than the same correlations in the juvenile trees (Tables 3.9 and 3.10). Reasons for weaker correlations among these traits in mature trees may be twofold. First, needle retention assessments in mature trees were conducted on previous year's needles which had not been affected as severely as older needles. In these trees, older needles make up a larger proportion of the foliage than one or two-year-old needles. Whereas in young trees all of the foliage is made up of one and two year-old needles, thus needle retentions and foliage density are more closely related in young trees than in older trees. Second, while one can observe the entire tree in a young plantation, visibility reduces as canopy closure increases with age. Difficulty in observing entire crowns in older plantations may have led to less reliable scoring of color and density.

Among the SNC traits in mature tests, needle retention had a significant strong and positive genetic relationship with all juvenile field traits, but with none of the laboratory traits, except with needle retention assessed in laboratory (Table 3.11). Mature foliage color was positively associated with juvenile needle color and foliage density, and was negatively associated with dry weight and needle length. There was no significant relationship between mature foliage density and juvenile traits (Table 3.11). The only juvenile laboratory traits associated significantly with mature traits (foliage color) were dry needle weight and needle length. Because seedling traits measured in the field (especially needle retention

Table 3.9. Family mean (above diagonal) and genetic (below diagonal) correlations of Swiss needle cast juvenile traits.

	Field				Laboratory					
Field	Needle color	Needle reten.	Foliage density	Foliage color	Needle color	Needle reten.	Dry weight	Needle length	Pseudo-thecia	Needle specific area
Needle color	--	0.54*	0.50*	0.86*	0.41*	0.44*	0.03	-0.06	-0.02	-0.21
Needle retention	**0.61**	--	0.91*	0.64*	0.27*	0.59*	0.04	0.14	0.28*	0.01
Foliage density	**0.56**	**0.95**	--	0.58*	0.20*	0.51*	-0.04	0.06	0.28*	0.06
Foliage color	**0.88**	**0.81**	**0.70**	--	0.36*	0.41*	0.06	-0.05	0.05	-0.18
Laboratory										
Needle color	**1.22**	**0.75**	**0.51**	**1.04**	--	0.41*	0.18	0.22*	0.22*	-0.34*
Needle retention	**0.80**	**1.09**	**0.79**	**0.63**	**0.98**	--	0.13	0.19*	0.31*	-0.19*
Dry weight	0.13	-0.02	-0.26	0.11	0.18	-0.22	--	0.80*	-0.23*	-0.58*
Needle length	-0.13	0.06	-0.03	-0.18	0.07	**0.10**	**0.66**	--	-0.04	-0.33*
Stomata with pseudothecia	0.16	**0.66**	**0.62**	0.31	**0.77**	**0.81**	**-0.45**	0.07	--	0.21*

Table 3.9 (Continued)

	Field				Laboratory					
	Needle color	Needle reten.	Foliage density	Foliage color	Needle color	Needle reten.	Dry weight	Needle length	Pseudo-thecia	Needle specific area
Needle specific area	**-0.37**	-0.01	0.10	-0.34	**-0.49**	0.01	**-1.00**	**0.23**	**0.86**	--

and foliage color) appear to be more consistently and strongly associated with SNC symptoms in mature tree (i.e., needle retention and foliage color), and are easier to measure than similar seedling traits in the laboratory, field assessments of seedling traits are the most useful for early testing purposes.

Table 3.10. Family mean (above diagonal) and genetic correlations of Swiss needle cast traits over the two mature sites.

	Needle retention	Foliage density	Foliage color
Needle retention	--	0.28*	0.60*
Foliage density	**0.49**	--	0.41*
Foliage color	**0.76**	**0.42**	--

in bold.

Estimates of genetic gain in tolerance of mature trees to the *P. gaeumannii* were generally stronger and more consistent when juvenile selections were based on field traits, rather than traits measured in the laboratory (Table 3.12). Both foliage color and needle retention in older trees subjected to SNC appear to be the most amenable to improvement by early testing. Selection for needle color in the seedlings at age-2 is estimated to be 52% as efficient in improving mature foliage color as selections delayed until the mature stage (ages 10 and 12). Selection for needle retention in seedlings, on the other hand, is expected to give the same gains in mature-tree needle retention as selection at the later age.

Table 3.11. Estimated juvenile-mature (Type-B) genetic correlations of Swiss needle cast traits.

Juvenile	Mature		
Field	Foliage Density	Foliage Color	Needle Retention
Needle color	0.19	**0.53***	0.75*
Needle retention	0.25	0.42	**0.75***
Foliage density	0.30	0.57*	0.63*
Foliage color	0.29	0.50	0.83*
Laboratory			
Needle color	-0.34	0.47	0.72
Needle retention	0.09	0.41	0.75*
Dry weight	-0.38	-0.72*	-0.13
Needle length	-0.27	-0.72*	-0.17
Pseudothecia	0.09	0.24	0.25
Needle specific area	0.15	0.40	0.10

comparable traits are shown in bold.

Table 3.12. Estimated genetic gain from early selection (as a proportion of population mean prior to selection) and the relative efficiency of early selection (gain in mature traits following juvenile selection / gain if selection is based on mature traits).

	Gain in mature traits			Relative efficiencies		
Juvenile traits	Foliage Density	Foliage Color	Needle Retention	Foliage Density	Foliage Color	Needle Retention
Needle Color	0.12	0.21	0.19	0.16	0.52	0.91
Needle Retention	0.18	0.19	0.21	0.23	0.45	1.01
Foliage Density	0.22	0.26	0.18	0.28	0.63	0.87
Foliage Color	0.19	0.20	0.21	0.24	0.49	1.02
Laboratory Traits						
Needle Color	-0.16	0.14	0.14	-0.20	0.34	0.64
Needle Retention	0.06	0.18	0.21	0.08	0.43	0.98
Dry weight	-0.22	-0.26	-0.03	-0.28	-0.63	-0.14
Needle Length	-0.16	-0.28	-0.04	-0.21	-0.67	-0.20
Stomata with Pseudothecia	0.05	0.09	0.06	0.07	0.22	0.28
Needle Specific Area	0.09	0.15	0.02	0.11	0.36	0.11

3.5 Discussion

Natural inoculation of seedlings that occurred in the filed 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.

The two artificial infection methods could theoretically manipulate the amount of incolum and environment more precisely, but they are more labor intensive and technically demanding. Natural inoculation is particularly desirable when very large numbers of families are to be tested. Another advantage of natural inoculation is that the testing is done under conditions more similar to what seedlings would experience in field. It is unclear the extent to which susceptibility or tolerance under artificial inoculation would translate to under actual field conditions. Furthermore, in this study artificial inoculations were not successful in that disease symptoms did not develop. Reasons for this may be twofold; the amount of spores was not sufficient or the warm dry climate at Dorena prohibited symptom development. Therefore, natural inoculation appears adequate for early testing purposes, but its efficiency needs to be verified in wider ranges of conditions.

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 be exposed to disease under more or less the same conditions.

Determination of appropriate traits to use in an early testing effort was one of the main objectives. In addition to the commonly used foliage traits in SNC assessments (e.g. color, density and retention) several traits were assessed in the laboratory. These traits are more expensive to assess than the field traits and do not improve efficiency of early selection. Therefore, none of these laboratory traits appear to be promising enough to replace the visually assessed foliage traits.

While severity of symptoms varied significantly among families, lack of significant family variation for fungal DNA content (even when large number of families investigated, 6 families in Chapter 2 versus 55 families in this study) is consistent with the hypothesis that tolerance is Douglas-fir's defense mechanism against SNC.

Early testing can be integrated into breeding programs in two ways: either early single-stage selection or multiple-stage selection. In single-stage selection,

superior genotypes are identified based on seedling performance in order to shorten generation intervals and increase genetic gain per unit of time. For this type of selection to be more efficient than mature selection in terms of gain per unit of time, relative efficiency of early selection should be larger than the ratio between generation intervals for early testing and mature testing. 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).

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, poor-performing genotypes 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 to 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 top 20% of the original population selected after two stages of selection.

Heritability and correlation estimates for SNC traits in this study equal or exceed the above values. Therefore, early selection can be integrated into current breeding programs in the region. Since the growth and wood quality are the most emphasized traits in current Douglas-fir breeding programs and increased number of traits reduces the efficiency of selection, single-stage early selection can be suggested in breeding for SNC tolerance.

3.6 Conclusions

While natural inoculation was sufficient for infection and symptom development in this study this method may not be feasible if very large number of families to be tested. In addition, natural inoculation may not produce desired infection and symptoms for evaluation in different environments. Suspending infected branches over seedlings in a growth chamber would allow large numbers of families to be tested and give breeders more flexibility in inoculation. In this study, infection was achieved by this method but the disease symptoms failed to develop, probably because of the dry climate at Dorena Tree Improvement Center during incubation. Therefore, optimum conditions for symptom development should be provided if this inoculation method to be employed.

Field assessments of the foliage traits (i.e., needle retention, needle color, foliage color and foliage density) seem sufficient for early testing purposes. These traits are under low to moderate genetic control and are related to each other at both juvenile and mature ages.

Early testing is particularly possible for needle retention and color due to high correlation between the two ages for these traits. While early selection for SNC tolerance can reduce breeding cycle but it is not practical under current breeding strategies for Douglas-fir because selection for the most important trait (i.e., growth and wood density) should be made a age 12-15, if the gain in these traits to be optimized. Early testing for SNC tolerance can still serve in two ways under current conditions. First, new families to be progeny tested can be screened for SNC tolerance and poor performing families 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.

Traits related to needle morphology and amount of fungal biomass in needles (i.e., laboratory traits) were also investigated in this study to see whether

they would better predict the family performance at the selection age than the visually assessed foliage traits would. None of the laboratory traits were sufficiently superior to visually assessed traits to justify their inclusion. Furthermore, while significant family differences were observed for foliage traits, amounts of fungal DNA in needles did not significantly differ among families. This finding supports the conclusion that Douglas-fir's mechanism against SNC is tolerance, not resistance.

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Chapter 4

Genetic Variation in Swiss Needle Cast Tolerance of Douglas-fir Along Climatic and Geographic Gradients in the Central Coast of Oregon

4.1 Abstract

The genetics of Swiss needle cast (SNC) tolerance, and relationships between climatic and geographic variables at mother tree locations and SNC tolerance of offspring, were investigated in 152 wind-pollinated Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) families originating from the central coast of Oregon in the Siuslaw National Forest. Two-year-old progeny were visually assessed for foliage greenness and proportion of retained foliage in two trials naturally inoculated with *Phaeocryptopus gaeumannii* spores released from the surrounding infected stands. Each character was scored at an individual internode (i.e., needle color, needle retention) and overall crown (i.e., foliage color, foliage density), making a total of 4 foliage traits. Genetic control over the four traits was weak as evidenced by the low heritability estimates ($0.11 \leq h_i^2 \leq 0.20$). Related traits appeared to be controlled by the same sets of genes with the estimated genetic correlation between color traits (r_A), 0.94, and retention traits, 0.96. Genetic correlations between color and retention traits were moderate but significant (mean = 0.60) suggesting that they have a number of controlling genes in common. Families performed similarly between the two trials, with Type-B genetic correlations exceeding 0.83. Family means for foliage retention and color were regressed on 9 geographic and climatic variables of mother tree locations to investigate their relationships with these variables. No significant associations were found between foliage retention and either geographic or climatic variables. There was some evidence, however, that the variation in foliage color is associated with elevation, aspect and distance to the Pacific Ocean of mother tree locations. Families originating from low elevations with southerly aspects are better able to maintain greener foliage, but the effect of aspect decreases in further north latitudes. The greenest foliage was observed in the sources located about 20 km from the coast and foliage color deteriorates from mother tree locations closer to

the coast or further inland. Although significant ($P < 0.05$), these variables explained only 14% of variation in foliage color.

4.2 Introduction

Swiss needle cast (SNC) is a fungal foliage disease of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco). Airborne ascospores of *Phaeocryptopus gaeumannii* [Rohde] Petrak infect newly emerging Douglas-fir needles in the spring through their stomatal openings. Infection and colonization of needles continue throughout the summer and fruiting bodies of the fungus (pseudothecia) emerge from stomata by the winter in the Oregon Coast Range (Capitano 1999), impairing gas exchange of needles and their ability to control water loss (Manter *et al.* 2000). Most of the infected needles eventually discolor and are cast prematurely resulting in sparse crowns and significant growth loss (Beekhuis 1978, Maguire *et al.* 2002).

Swiss needle cast was first reported in Switzerland (Gäumann 1930) and later reports came from New Zealand (Hood and Kershaw 1975), Australia (Hood 1997) and Germany (Boyce 1940, Merkle 1951) where Douglas-fir has been planted as an exotic. Damaging effects of SNC were also reported from the Lake States (Morton and Patton 1970) and Pennsylvania (Merrill and Longenecker 1973) in the United States of America. Although the fungus causing the disease is native to the Pacific Northwest region of the United States and has been known to foresters since early 1900's (Boyce 1940), the disease has not been considered damaging or problematic until recently. Swiss needle cast was first reported as a problem in Christmas tree plantations in Oregon where fungicides are often used to alleviate disease symptoms (Hadfield and Douglas 1982). In late 1980's severe symptoms of SNC were also reported in older, forest plantations of coastal

Douglas-fir (var. *menziesii*) around Tillamook, Oregon (Kanaskie *et al.* 1996, Hansen *et al.* 2000).

Several explanations have been proposed for this seemingly sudden appearance of severe SNC symptoms in coastal Oregon and Washington: (1) evolution of a new virulent *P. gaeumannii* strain, (2) increased disease pressure as a result of establishing Douglas-fir plantations in disease prone areas, and (3) use of inappropriate Douglas-fir seed sources in coastal plantations.

Winton (2001) investigated the genetic variation in *P. gaeumannii,* using single-strand conformational polymorphisms (SSCP, Orita *et al.* 1989) to screen for DNA sequence variation at five loci in the *P. gaeumannii* genome, on samples obtained from 30 populations, both within the natural range of Douglas-fir and where the species is planted as an exotic. Two reproductively isolated sympatric lineages were found in the region of Oregon's Swiss needle cast epidemic. While one of the lineages had a worldwide distribution, occurring throughout much of the Pacific Northwest as well as locations where Douglas-fir had been planted as an exotic, the other lineage was restricted to coastal Oregon. A positive correlation was observed between the severity of SNC symptoms and abundance of the second lineage in the region.

Increased forestry activities in the *Picea sitchensis* vegetation zone (Franklin and Dyrness 1973) resulted in stands being converted to plantations of the more valuable and faster growing Douglas-fir. The *Picea sitchensis* vegetation zone is a narrow band of coastal forests that stretch from south-east Alaska to northern California. It is suspected that frequent fog occurrence, mild temperatures and high humidity in this zone -due to low elevation, high precipitation (> 300 cm yr^{-1}), and proximity of this zone to the Pacific ocean- is conducive to infection by *P. gaeumannii*. In addition, Douglas-fir plantations established in the area provided abundant foliage that served as a large breeding ground for the fungus (Hansen *et al.* 2000), increasing the available inoculum in the air and the disease pressure.

Maladaptation may occur when non-local seed sources are employed to establish new plantations (Adams and Campbell 1980). This is presumably because genetic variation is a direct result of environmental variation responsible for natural selection; thus, a population of a given species may be better adapted to its place of origin than any other population (Campbell 1986). Therefore, more genetic difference and thus higher risk of seed transfer is expected with increased distance between origin of a seed source and planting location. Like many other conifer forest tree species, seed production in coastal Douglas-fir is unreliable because of irregular seed years (Fenner 1991). Thus, at least some of the Douglas-fir plantations established in the *Picea sitchensis* vegetation zone may have come from non-local seed sources- especially sources from higher elevations and more inland where seed production is more reliable. Non-local seed sources may not be well adapted to the local climate conditions and thus, are more susceptible to *P. gaeumannii* than native seed sources. While any of the factors mentioned may be contributing, it is highly unlikely that any one of them individually is responsible for the current outbreak of severe SNC symptoms; rather, it is probably a combination of these factors (Hansen *et al.* 2000).

No individual trees appear to be immune from SNC symptoms in the region where the disease is epidemic. Nevertheless, there are differences in the degree of expression of disease symptoms, even between trees located close together. Some of this variation is due to genetic differentiation in tolerance to the disease (Chapters 2 and 3 of this thesis). Presence of genetic variation in the expression of SNC symptoms in Douglas-fir has been documented for full-sib families of Christmas trees (Nelson *et al.* 1989), clones (Blada 1988), wind-pollinated families (Johnson 2002, Chapters 2 and 3 of this thesis) and at the provenance level (Hood and Wilcox 1971 (unpublished), McDermott and Robinson 1989, Stephan 1997).

Geographic variation in growth and bud phenology are well documented in coastal Douglas-fir. Variation in these traits is closely associated with temperature, moisture, latitude, elevation and distance from the Pacific Ocean of seed source

locations (Campbell and Sorensen 1978, Campbell 1979, Campbell 1986). Johnson and Temel (1999 Swiss Needle Cast Cooperative Annual Report, Oregon State University, Corvallis, Oregon), using a subset of families included in this study, reported higher foliage retention for SNC-infected families that originated from areas closer to the coast. McDermott and Robinson (1989) examined a provenance trial in British Columbia and found more tolerance to SNC in provenances from high rainfall areas than from low rainfall areas. There is no study reported in the literature, however, investigating variation in SNC tolerance in relation to geographic and climatic factors of seed source locations in coastal Oregon.

In this study, genetic and geographic variation in tolerance of Douglas-fir to SNC was investigated. Swiss needle cast symptoms were examined on two-year-old trees from 152 wind-pollinated Douglas-fir families originating from the central Oregon coast. SNC tolerance of families was related to geographic location and climate variables at mother tree locations by regression analysis.

4.3 Material and Methods

4.3.1 Origin of Materials

The 152 wind-pollinated Douglas-fir families used in this study originated from mother trees located in the Siuslaw National Forest. The Siuslaw National Forest constitutes 255,150 ha of diverse forest, with elevation ranging from sea level to 1,249 m (Figure 4.1). The wind-pollinated families came from stored seed-lots of the mother trees originally selected for U.S. Forest Service tree improvement programs. For the purposes of this study, families were specifically chosen so that their mother trees were distributed as wide and as uniform as possible across the region (Figure 4.1). Elevation of mother trees ranged between 25 and 667 m (mean = 331 m) and distance to the Pacific Ocean between 1 and

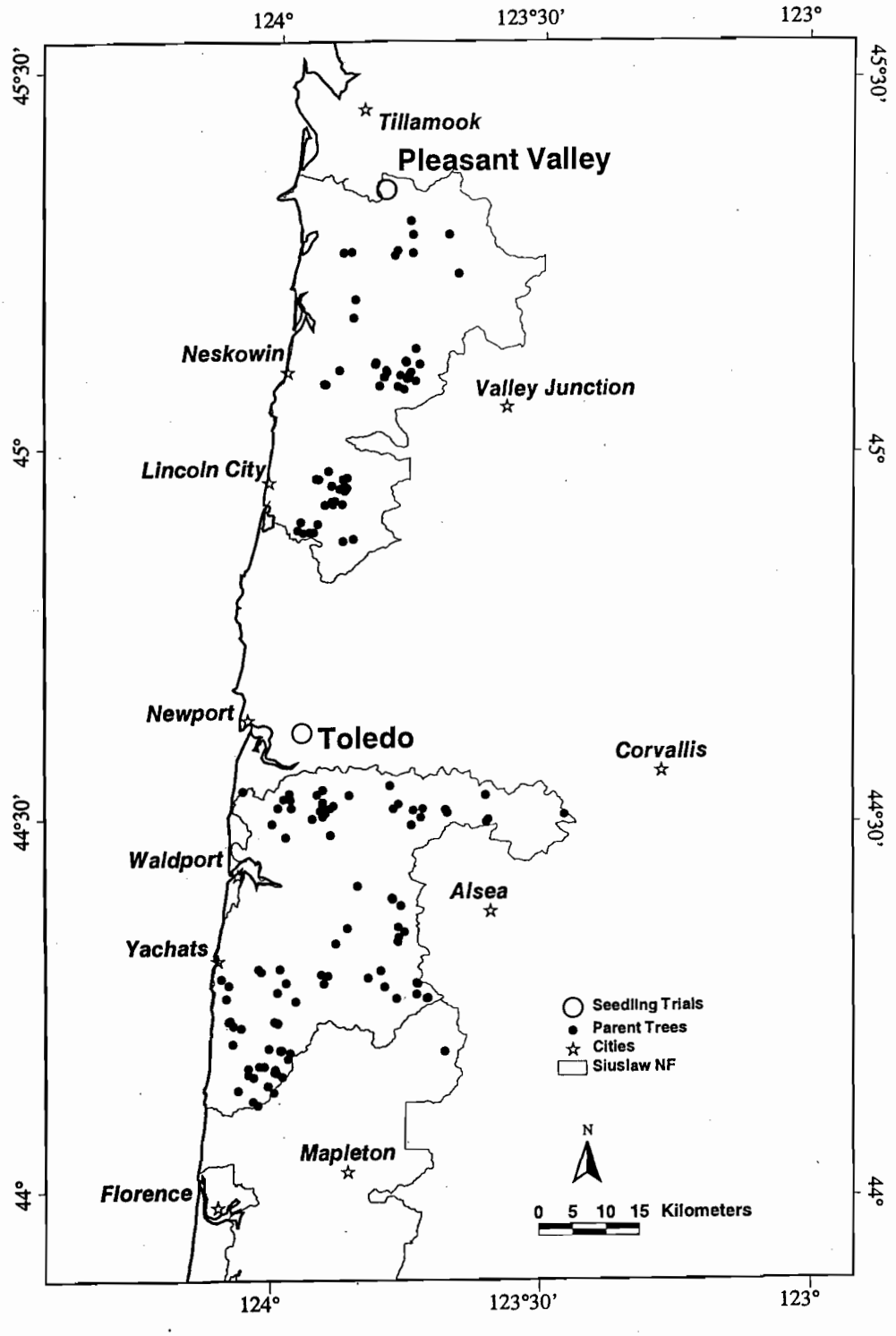

Figure 4.1. Locations of 152 mother trees and seedling trials in coastal Oregon.

48.9 km (mean = 15.2 km). Only seven locations (4.6%) were on a flat surface (no aspect); mother trees were located on slopes as steep as 65.3% (mean = 20.3%). South and west aspects were dominant among the locations (100 locations, or 65.8%); north and east aspects constituted 29.6% (45 locations).

4.3.2 Seedling Stock Development

Plant material to establish the seedling trials was grown at the Dorena Tree Improvement Center near Cottage Grove, Oregon. Seeds, collected in 1985 and stored in the freezer, were soaked in 1% H_2O_2 for 24 h, soaked in water for an additional 24 h and drained, and then stratified for 60 d at 0.6 - 1.7 °C. Visual inspection of the seeds was carried out weekly for moisture and mold. Moldy seeds were rinsed thoroughly with 1% H_2O_2.

Stratified seeds were sown in 164 ml Ray Leach Supercells (Stuewe & Sons Inc., Corvallis, OR) (2 seeds per cell) on the 17th and 18th March 1998. Sowing media consisted of peat:vermiculite:composted fir bark:perlite:pumice (40:30:10:10:10). The cells were filled to approximately 75% volume with the media and 60 g of Nutricote controlled release fertilizer (180 d release time, 18% N, 6% P and 8% K) with minor elements (1.2% Mg, 0.02% B, 0.05% Cu, 0.2% Fe, 0.06% Mn, 0.02% Mo, and 0.015% Zn) applied to each cell (0.6 g/cell). The cells then were completely filled and watered prior to sowing. Seeds were hand sown in depressions made in the surface of the cells and covered with a thin layer of grit (< 7 mm).

Each family was kept in a separate supercell rack for convenience. There were 98 cells (7 rows by 14 columns) per rack. A total of 140 cells were sown per family (20 columns, or approximately 1.5 racks per family). The racks were placed in a greenhouse after sowing and covered with screens to protect seeds from birds

and rodents. The soil surface was kept moist and the greenhouse temperature was kept below 30 °C throughout the germination period.

After germination was completed, the seedlings in each cell were randomly thinned to leave one seedling per cell. Later, the seedlings were moved outside on raised beds where they were randomly assigned into replications. The first seedling in each column of seedlings in each rack was tagged with family information and replication number (from 1 to 20). Then the first 5 seedlings starting from the tagged seedling in each of the 20 columns were randomly assigned to 20 replications. Each replication was a complete block of 152 families where each family was represented by a 5-seedling row plot (three family plots in each row). A border row of seedlings was placed around each replication using the remaining seedlings. Replications were kept in this arrangement until transplanting. The integrity of these replications, at least in composition of families and row plots, was maintained throughout in remainder of the study.

The soil surface was kept moist during the growing season until mid-July. After the primary needles emerged the seedlings were watered as needed. Dormancy was induced in late August by discontinuing regular irrigation. During the dormant season, irrigation was applied to field capacity when soil tension reached – 0.7 bars.

In addition to fertilization at sowing, the following fertilization regime was applied during the growing season starting after emergence was completed.

Weeks 1-3 (May 4- May 22). 100 ppm Peter's Excel 15-5-15 with Ca + Mg applied once/week. One fertilization with 80 ppm Peter's Conifer Grower (20-7-19) replaced the Excel during this period.

Weeks 4-8 (May 25 – June 26). 150 ppm Peter's Excel 21-5-20 with Ca + Mg once per week. Fe supplemented 1-2 fertilizations.

Weeks 9-12 (June 29 – July 24). 100-125 ppm Peter's Conifer Grower (20-7-19) or Plantex All Purpose (20-20-20) every 10 days.

Weeks 13-19 (July 27 – Sept. 4). 150-200 ppm Peter's Excel (21-5-20) with Ca + Mg alternated with 150 ppm Peter's Conifer Grower every 7-10 days. Fe supplemented 2-3 fertilizations.

Leach heavily at week 19. Starting in mid to late September, 100 ppm Peter's Conifer Finisher (4-25-35), or similar low N, high P and K fertilizer, was applied every 7-10 days through the end of October.

On 5 January 1999, 16 of the 20 replications were put in cold storage, generously provided by The Timber Company (now part of Plum Creek Co.), near Cottage Grove, Oregon. Justification for this treatment is as follows: In natural populations of infected Douglas-fir, maximum spore release occurs in June (Michaels and Chastagner 1984). While newly emerged needles are more susceptible to infection than older needles (Stone *et al.* 1998 SNCC Annual Report), there is normally a great deal of variation in bud-burst timing in this species (Li and Adams 1993). Thus, in order to have effective artificial inoculation in the field, uniform timing of budbreak among seedlings is important. Chilling of seedlings is an effective way of controlling bud-burst timing in Douglas-fir (Campbell and Sugano 1975). Thus, seedlings employed in establishing the seedling trials were chilled in cold storage at 4 °C for 114 d (until 29 April 1999). Eight of these 16 replications were used to establish the seedling trials assessed in this study. The remainder of the replications was used in other experiments (Chapter 3 of this thesis).

4.3.3 Test Establishment and Maintenance

Two seedling trials, one in Toledo (planted on 12 May 1999), near Newport Oregon, and the other in Pleasant Valley (planted on 14 May 1999), near Tillamook Oregon, were established using the 1-year-old container grown seedlings (Table

4.1). Both plantations were located on Simpson Timber Company land near Douglas-fir stands heavily infected with *P. gaeumannii*.

A rectangular area about 300 m^2 was prepared for the seedling trials at the two locations. The land at Pleasant Valley had recently been planted with western hemlock (*Tsuga canadiensis*) and there was no competing vegetation. At Toledo the area had been cleared of Douglas-fir, salmonberry (*Rubus spectabilis*) and blackberry (*Rubus fruticosa*) using a root rake prior to planting.

Table 4.1. Information on seedling trials used in this study.

	Progeny Plantation	
	Pleasant Valley	Toledo
Elevation (m)	62	110
Latitude	45° 21' 04" N	44° 37' 12" N
Longitude	123° 48' 08" W	123° 57' 45" W
Aspect	West	North, Northwest

Following planting, the competing vegetation was removed by hand in Pleasant Valley (primarily grass) and by herbicide in Toledo (primarily *Rubus* sp.). Remnants of thick competing vegetation 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*. At the time of assessments, a total of 1597 trees were affected and 135 trees killed by gray mould (total number of dead trees = 230, 92.4% survival) in Toledo. Only 76 trees were lost in Pleasant Valley (97.5% survival).

It was felt that the stands around these seedling tests would provide a reliable source of inoculum. The statistical design was a randomized complete

block with 4 replications in each plantation, with each family represented by a 5-tree row plot in each replication. Within row spacing was 33 cm at both sites. Spacing between rows was 66 cm at Pleasant Valley and 50 cm at Toledo. The test sites were fenced to prevent damage by deer and elk.

4.3.4 Traits and Assessment Methods

In the summer of 2000 (one year after establishment; seedlings 2 years old from seed), the tests were visually assessed for four foliage traits representing SNC symptoms. **Needle color** was scored from 1 (yellow) to 3 (dark green) on the needle cohort produced during the 1999 growing season (i.e., 2 year-old foliage) on a single south facing branch. The same scale was used for **foliage color** assessment but the score was based on the entire crown of the tree. **Needle retention** was scored from 0 (10% retention) to 9 (> 90% needle retention) on the same needle cohort and branch scored for color. **Foliage density** was the overall appearance of all the foliage and was scored from 1 (sparse) to 6 (dense).

Geographic and climatic variables. Environmental factors play an important role in variation in severity of disease symptoms. Certain temperature and humidity conditions promote spore release (Michaels and Chastagner 1984) and provide better conditions for infection and colonization of Douglas-fir needles (Capitano 1999, Manter *et al.* 2001 SNCC Annual Report). SNC symptoms were found to be more severe at valley bottoms with southerly aspects, cooler summers and warmer falls, and high precipitation (Rosso and Hansen 1999 SNCC Annual Report). If the disease has been applying sufficient selection pressure on Douglas-fir in such disease conducive environments, one would expect seed sources from these locations to be more tolerant to SNC than the seed sources from unfavorable locations for the disease. If this is the case, variation in SNC tolerance can be related to geographic and climatic variation of seed sources. Thus, geographic and

climatic variables at mother tree locations were those related to temperature and moisture regimes, as temperature and moisture play a significant role in fungal infection and growth in Douglas-fir needles (Manter *et al.* 2001).

A total of 6 geographic and 8 climatic variables at mother-tree locations that might be related to variation in SNC severity were considered. The six location variables utilized were: latitude (Universal Transverse Mercator, UTM, coordinates), elevation (m), slope (%), aspect (2 variables), and distance to the Pacific Ocean (km). Aspect was measured as clock-wise deviation from north (0°) in degrees and was transformed into two variables that contrasted (1) east versus west aspects (sine transformation, where $\sin(90°) = 1$ and $\sin(270°) = -1$) and (2) north versus south aspects (cosine transformation, where $\cos(180°) = -1$ and $\cos(360°) = 1$) (Stage 1976). Distance to the Pacific Ocean is preferred over longitude, per se, because it better represents changes in the influence of the Pacific Ocean, because the longitude of the coast line varies (Figure 4.1).

Climatic variables were estimated for mother tree locations (UTM) using two different models and the Geographic Information System (GIS). 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.

Temperature and precipitation were selected because of their biological significance has been well documented in infection and symptom development. Evaporation, potential evapotranspiration and transpiration are related to humidity surrounding Douglas-fir needles, which is critical for *P. gaeumannii* infection. Months of June and November were found to be epidemiologially relevant in SNC (Capitano 1999) and climatic conditions in these months were found to be associated with SNC severity in Coastal Oregon (Rosso and Hansen 1999 SNCC Annual Report). Thus, maximum and minimum average temperatures,

precipitations and potential evapotranspiration rates for June and November were considered. As a result, a total of 14 geographic and climatic variables were considered in regression models (Table 4.2).

Table 4.2. Means and ranges of the 14 geographic and 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)

4.3.5 Data Analysis

All four foliage traits were subjected to analysis of variance (ANOVA) to determine significance of differences among families. Differences were deemed

significant when $P < 0.05$. The SAS statistical software package (SAS Institute 1990) was used for all calculations.

ANOVA was conducted on the data for each trial site according to the following model:

$$y_{jkl} = \mu + r_j + f_k + rf_{kj} + e_{jkl}$$

where, y_{jkl} is the l_{th} tree from the k_{th} family in the j_{th} replication; μ is overall mean; r_j is the effect of the j_{th} replication, $E(r_j) = 0$, $Var(r_j) = \sigma_r^2$; f_k is the effect of the k_{th} family, $E(f_k) = 0$, $Var(f_k) = \sigma_f^2$; rf_{kj} is the effect of interaction between the j_{th} replication and the k_{th} family, $E(rf_{kj}) = 0$, $Var(rf_{kj}) = \sigma_{rf}^2$; and e_{jkl} is the within plot error, $E(e_{jkl}) = 0$, $Var(e_{jkl}) = \sigma_e^2$.

Error variances were consistent for each trait from site to site in each group, so there was no adjustment necessary to combine the data over sites (Appendix C). The ANOVA for the combined data set over the two sites was:

$$y_{ijkl} = \mu + s_i + r_{j(i)} + f_k + sf_{ik} + rf(s)_{jk(i)} + e_{ijkl}$$

where, y_{ijkl} is the l_{th} tree from the k_{th} family in the j_{th} replication in the i_{th} site; μ is the overall mean; s_i is the effect of the i_{th} site, $E(s_i) = 0$, $Var(s_i) = \sigma_s^2$; $r_{j(i)}$ is the effect of the j_{th} replication in the i_{th} site, $E(r_{j(i)}) = 0$, $Var(r_{j(i)}) = \sigma_{r(s)}^2$; f_k is the effect of the k_{th} family, $E(f_k) = 0$, $Var(f_k) = \sigma_f^2$; sf_{ik} is the interaction between the i_{th} site and the k_{th} family, $E(sf_{ij}) = 0$, $Var(sf_{ij}) = \sigma_{sf}^2$; $rf(s)_{jk(i)}$ is the interaction between the k_{th} family and the j_{th} replication in the i_{th} site (or, plot error), $E(rf(s)_{jk(i)}) = 0$, $Var(rf(s)_{jk(i)}) = \sigma_{rf(s)}^2$; and e_{ijkl} is the random error (or, within plot error), $E(e_{ijkl}) = 0$, $Var(e_{ijkl}) = \sigma_e^2$.

Analyses of variance were conducted to test significance of site and family differences using the PROC GLM (generalized linear models) of SAS (Type III sums of squares). Forms of the expected mean squares are given in Table 4.3. The

REML (restricted maximum likelihood) method of the VARCOMP procedure was used to estimate variance components. REML estimates of variance components are more reliable than ANOVA estimates when imbalance exists in data (Swallow and Monahan 1984, Searle *et al.* 1992, White 1996).

Table 4.3. Form of ANOVA and expected mean squares for the seedling tests. A) For individual trial sites, and B) for combined data from the two sites [a)].

Source	Degrees of freedom	Expected Mean Squares
A) Individual Trials		
Replication	r-1	$\sigma_e^2 + t\sigma_{rf}^2 + tf\sigma_r^2$
Family	f-1	$\sigma_e^2 + t\sigma_{rf}^2 + tr\sigma_f^2$
Rep.*Family	(r-1)(f-1)	$\sigma_e^2 + t\sigma_{rf}^2$
Within Plot Error	(t-1)rf	σ_e^2
B) Both Trials Combined		
Site	s-1	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tr\sigma_{sf}^2 + tf\sigma_{r(s)}^2 + trf\sigma_s^2$
Replication(Site)	(r-1)s	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tf\sigma_{r(s)}^2$
Family	f-1	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tr\sigma_{sf}^2 + trs\sigma_f^2$
Site*Family	(s-1)(f-1)	$\sigma_e^2 + t\sigma_{rf(s)}^2 + tr\sigma_{sf}^2$
Family*Rep.(Site)	(r-1)(f-1)s	$\sigma_e^2 + t\sigma_{rf(s)}^2$
Within Plot Error	(t-1)rfs	σ_e^2

[a)] Assumes no missing plots in the original design. s = number of sites, r = number of replications per site, f = number of families, t = number of trees per plot, σ_e^2 = within plot error, $\sigma_{rf(s)}^2$ = variation due to replication-by-family interaction, σ_{sf}^2 = variation due to site-by-family interaction, σ_f^2 = variation due to differences between families, $\sigma_{r(s)}^2$ = variation due to replications, and σ_s^2 = variation due to sites.

To determine the significance of site differences, the denominator degrees of freedom (*ddf*) was approximated following Satterthwaite (1946) as cited in Milliken and Johnson (1984, pages 250-251):

$$ddf = \frac{[MSR(S) + MSSF - MSRF(S)]^2}{\frac{[MSR(S)]^2}{(r-1)s} + \frac{[MSSF]^2}{(s-1)(f-1)} + \frac{[MSRF(S)]^2}{(r-1)(f-1)s}},$$

where *MSR(S), MSSF* and *MSRF(S)* are mean squares for replication(site), site*family, and the replication*family(site) components, respectively (also see Table 4.3).

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 correlations among traits. Additive genetic variance (σ_A^2) was estimated as four times the family component of variance (σ_f^2), and assumes individuals in these families are true half-sibs. Total phenotypic variation was estimated as, $\sigma_{PI}^2 = \sigma_e^2 + \sigma_{sf}^2 + \sigma_{rf(s)}^2 + \sigma_f^2$ for the combined data set over the two trials and as $\sigma_{PI}^2 = \sigma_e^2 + \sigma_{rf}^2 + \sigma_f^2$ for the individual trials. Total phenotypic variation of family means was estimated as

$\sigma_{PF}^2 = \frac{\sigma_e^2}{rt} + \frac{\sigma_{sf}^2}{s} + \frac{\sigma_{rf(s)}^2}{r} + \sigma_f^2$ for the combined data set and as

$\sigma_{PF}^2 = \frac{\sigma_e^2}{rt} + \frac{\sigma_{rf}^2}{r} + \sigma_f^2$ for individual trials (see table 4.3 for an explanation of terms).

Due to the imbalance in the data, r (number of replications per site) and t (number of trees per plot) used in above analyses were given by the GLM procedure.

Narrow-sense individual (h_i^2) and family (h_f^2) heritabilities were estimated following Falconer (1981):

$$h_i^2 = \frac{\sigma_A^2}{\sigma_{PI}^2} \text{ and } h_f^2 = \frac{\sigma_F^2}{\sigma_{PF}^2} = \frac{\frac{1}{4}\sigma_A^2}{\sigma_{PF}^2}.$$

Standard errors of heritability estimates were approximated following Dickerson (1969):

$$SE(h_i^2) = SE(\frac{4\sigma_F^2}{\sigma_{PI}^2}) = \frac{C}{\sigma_{PI}^2}\sqrt{\text{var}(\sigma_F^2)},$$

$$SE(h_f^2) = SE(\frac{\sigma_F^2}{\sigma_{PF}^2}) = \frac{C}{\sigma_{PF}^2}\sqrt{\text{var}(\sigma_F^2)},$$

where, C is the respective coefficient of σ_F^2 in estimating heritabilities and $\text{var}(\sigma_F^2)$ is given by the REML. These approximations of standard errors are conservative (i.e., slightly inflated) because terms involving $\text{var}(\sigma_{PI}^2)$ (or $\text{var}(\sigma_{PF}^2)$) and $Cov(\sigma_F^2\sigma_{PI}^2)$ (or $Cov(\sigma_F^2\sigma_{PF}^2)$) are neglected.

Additive genetic correlations among traits were estimated as

$$r_A = \frac{Cov_F(x,y)}{\sqrt{\sigma_{Fx}^2 \cdot \sigma_{Fy}^2}},$$

where r_A is additive genetic correlation between traits x and y, $Cov_F(x,y)$ is the family covariance between traits x and y, estimated as $Cov_F(x,y) = \frac{\sigma_{F(x+y)}^2 - \sigma_{Fx}^2 - \sigma_{Fy}^2}{2}$, and σ_F^2 is the respective family variance components for traits x, y and their sum.

Type-B genetic correlations between the sites were estimated following Burdon (1977):

$$r_B = \frac{r_{xy}}{\sqrt{h_{fx}^2 . h_{fy}^2}},$$

where, r_B is the genetic correlation for a given trait between the Pleasant Valley (x) and Toledo (y) trials; r_{xy} is the family mean correlation for the given trait between

the trials; h^2_{fx} and h^2_{fy} are the estimates of family mean heritabilities for the given trait at Pleasant Valley and Toledo, respectively. The variance components used in genetic correlation calculations were obtained from data standardized by subtracting site mean and dividing by the site's phenotypic standard deviation (Johnson *et al.* 1997).

To investigate the relationships between foliage traits (needle retention, needle color, foliage color and foliage density) and geography and climate of mother tree locations, data analyses were conducted in two steps. First, the number of variables in Table 4.2 was reduced to 9 by investigating the relationships among these variables. A correlation analysis was conducted and the variable pairs with correlations larger than |0.70| were determined. These were: Distance to the Pacific Ocean and June average maximum temperature (0.76); Latitude and June potential evapotranspiration (-0.76); Latitude and November average maximum temperature (-0.79); June average minimum temperature and November average maximum temperature (0.94); June average minimum temperature and November average minimum temperature (0.96); and November average maximum and minimum temperatures (0.91). As a result, June average maximum temperature, June potential evapotranspiration rate, November average maximum temperature, and November average minimum temperature were removed from the variable list. Furthermore, from the two aspect variables (sine and cosine) only the one that indicates southerly aspects (cosine of aspect) was kept in the list. Thus, the following 9 location variables were considered in the regression analysis: distance to the Pacific Ocean, elevation, latitude, slope, cosine of aspect, June average precipitation, June average minimum temperature, November average precipitation, and November potential evapotranspiration. In addition, squares of Distance to the Pacific Ocean and elevation were included as additional independent variables to account for curvilinear relationships between these variables and SNC traits (Campbell and Sorensen 1978, Sorensen 1983, Balduman *et al.* 1999). A total of 5 interactions between location variables were also included to observe the changes

in SNC severity with the combined effects of the location variables. Considered interactions were: distance to the Pacific Ocean and elevation, latitude and elevation, distance to the Pacific Ocean and cosine of aspect, elevation and cosine of aspect, and latitude and cosine of aspect. Thus, a total of 16 independent variables were used in the original "full" models tested by regression analysis.

In the second step, the foliage traits were regressed over the 16 independent variables. The RSQUARE and the Cp options of the PROC REG procedure of SAS were used in determining a final model. The RSQUARE option lists all regression models from largest to smallest R^2 for a given number of independent variables in the model. The Cp statistic is proposed by Mallows (1973) as a criterion for selecting a model. It is a measure of total squared error defined as

$$Cp = \frac{SSEp}{s^2} - N + 2p$$

where s^2 is the mean square error for the full model, and *SSEp* is the sum-of-squares error and N is the error degrees of freedom for a model with p parameters including the intercept, if any. If Cp is plotted against p, Mallows recommends the model where Cp first approaches p. When the right model is chosen, the parameter estimates are unbiased, and this is reflected in Cp near p.

4.4 Results and Discussion

4.4.1 Genetics of Foliage Traits

Survival of seedlings two years after transplanting was better at Pleasant Valley (97%) than at Toledo (87%). There are several factors contributing to this. The Pleasant Valley trial was established on a piece of land that was previously planted with western hemlock (*Tsuga canadiensis*). Competing vegetation had been controlled for western hemlock at the time of planting, thus there was no

significant competing vegetation at the site. At Toledo, however, a patch of land was cleared just before planting. There was a significant amount of dead organic matter on the ground and a large stump on the site. Soil disturbance caused by the heavy machinery was still visible at the time of planting. Although competing vegetation (primarily salmonberry [*Rubus spectabilis*] and blackberry [*Rubus fruticosa*]) was removed prior to test establishment, it re-claimed the land in the following years. Physical removal of competing vegetation was sufficient at Pleasant Valley, but additional herbicide applications were necessary at Toledo. Remnants of competing vegetation and dead organic matter already present at Toledo reduced air movement around trees, creating ever present wet conditions and thus predisposing the trees to a common fungal nursery disease, gray mould, caused by *Botrytis cinerea*. In fact, almost all of the casualties suffered at Toledo (94%) were due to gray mould.

On average, SNC disease symptoms were more severe at Pleasant Valley than at Toledo (Table 4.4). Site difference, however, was statistically significant only for needle and foliage color. Although measurements were not taken to quantify inoculum densities, difference in inoculum load was probably not the reason for the observed difference in symptom severity between the sites. In an investigation conducted on a subset of the families (n = 55), neither families nor sites were found to differ in the mean amount of fungal biomass in sampled needles (Chapter 2 of this thesis). Assuming that higher inoculum density results in more infection, and more fungal biomass in needles, it is likely that inoculum loads did not differ between sites. Pleasant Valley is located in an area where the disease symptoms are most prevalent in the region. The reason for higher symptom severity at Pleasant Valley, therefore, can simply be a result of its location.

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. Tree-to-tree variation within plots accounted for the bulk (82.82% to 90.85%) of the total

variation (Table 4.5). Although the SNC trait assessments at Toledo were confounded by the damage caused by *Botrytis cinerea*, family rankings were consistent between the trials. Family mean correlations for the same traits between the two sites were significant and genetic correlations very high (Table 4.6), indicating that ranking of families was not affected by *B. cinerea* damage.

Table 4.4. Overall means, family mean ranges (in parentheses) and standard deviations (SD) of family means for Swiss needle cast traits.

	Pleasant Valley		Toledo	
	Mean	SD	Mean	SD
Needle Color*	1.75 (1.20-2.45)	0.22	1.99 (1.47-2.55)	0.22
Foliage Color*	1.82 (1.15-2.40)	0.21	2.09 (1.54-2.75)	0.21
Needle Retention	7.40 (3.30-8.75)	0.84	8.30 (5.69-9.00)	0.54
Foliage Density	4.39 (2.27-5.65)	0.59	4.89 (3.39-5.75)	0.46

*) Significant site differences.

Foliage traits were weakly heritable, with individual narrow-sense heritability estimates ranging from 0.11 to 0.20. Family mean narrow-sense 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) (Table 4.7). Heritability estimates were higher at Pleasant Valley than at Toledo, especially for needle retention and foliage density. Lower heritability estimates at Toledo may be due to the *B. cinerea* infection (60% of surviving trees) that caused loss of needles and/or leaders, thus confounding

Table 4.5. Estimated test means for data combined over trials (range over family means in parentheses), variance components (expressed as intraclass correlation coefficients; calculated as ratio of individual variance component estimate to sum of the all components), and total phenotypic variances for four Swiss needle cast traits included in this study.

		Variance Components [a] (%)				
	Means	F	S*F	P	WP	Phenotypic Variance
Needle Color	1.87 (1.50-2.41)	2.76	0.66	5.70	90.88	0.5088
Foliage Color	1.95 (1.50-2.38)	3.60	0.90	8.31	87.19	0.4001
Needle Retention	7.85 (4.71-8.80)	4.93	0.17	10.14	84.76	4.0965
Foliage Density	4.64 (2.88-5.68)	4.89	0.15	12.16	82.80	2.2513

[a] F = Family, S*F = Site by Family, P = Plot, WP = Within Plot.

assessments of needle retention and foliage density associations with *P. gaeumannii*.

Although low, the magnitude of the heritability estimates for the SNC traits are not unusual for traits assessed subjectively. For equivalent SNC foliage traits Johnson (2002) reported similar estimates of narrow-sense heritabilities based on data from seven 11-year-old wind-pollinated Douglas-fir progeny test sites. Average heritability estimates in Johnson's (2002) study were 0.17 (range 0.04 to 0.30) for foliage color, 0.16 (range 0.03 to 0.28) for needle retention and 0.25 (range 0.16 to 0.44) for foliage density. The low heritabilies may also be due to the subjective nature of assessments, although the same person conducted SNC assessments at both sites in the current study. In addition, all traits are categorical and thus are more prone to violations of the normality assumption of ANOVA.

Table 4.6. Genetic (above diagonal, bold) and family mean (below diagonal, regular) correlations among Swiss needle cast traits and family mean and Type-B genetic correlations for the same traits between the two sites [a)].

	Between traits				Between sites	
	Needle Color	Foliage Color	Needle Retention	Foliage Density	Family mean	Type-B Genetic
Needle Color	--	**0.94**	**0.52**	**0.53**	0.29	0.83
Foliage Color	0.87	--	**0.68**	**0.62**	0.33	0.84
Needle Ret.	0.46	0.57	--	**0.96**	0.44	1.13
Foliage Dens.	0.44	0.52	0.88	--	0.41	1.21

[a)] All family mean correlations are significant at P = 0.05 level. Data were pooled over the two trials.

Table 4.7. Individual (I) and family mean (F) narrow-sense heritability estimates for Swiss needle cast traits (standard errors of the estimates are in parentheses).

	Pleasant Valley		Toledo		Combined	
	I	F	I	F	I	F
Needle Color	0.15 *(0.046)*	0.39 *(0.122)*	0.12 *(0.049)*	0.31 *(0.124)*	0.11 *(0.033)*	0.44 *(0.132)*
Foliage Color	0.21 *(0.055)*	0.46 *(0.120)*	0.14 *(0.053)*	0.33 *(0.122)*	0.14 *(0.038)*	0.48 *(0.128)*
Needle Retention	0.28 *(0.068)*	0.49 *(0.117)*	0.12 *(0.048)*	0.31 *(0.125)*	0.19 *(0.043)*	0.54 *(0.123)*
Foliage Density	0.31 *(0.069)*	0.52 *(0.115)*	0.09 *(0.050)*	0.22 *(0.127)*	0.20 *(0.043)*	0.56 *(0.121)*

Estimated family mean correlations for all pairs of SNC traits were fairly moderate to strong and statistically significant. Genetic correlations between the

two "color" traits and between the two "retention" traits were both near unity (Table 4.6), indicating that the similar traits in each pair are essentially under the same genetic control. Thus it makes little difference if the entire seedling or just one internode is evaluated for these traits. Genetic correlations between needle color and density traits were lower, but still strong enough to suggest that SNC tolerance genes expressed in foliage color and retention largely overlap.

Johnson (2002) observed relationships between foliage color and density traits in 11-year-old trees (mean $r_g = 0.60$) similar to this report. The genetic correlation between foliage density and needle retention was, however, much smaller in the older trees (mean = 0.26) than what was observed in this study (0.96). Difference in tree ages between the two studies (11- versus 2-year-old) can explain this difference in results. While needle retention is assessed on 1- and 2-year-old needles, foliage density is based on the entire crown. Typically, healthy Douglas-fir maintain at least 5 years of needles (Hood 1982). Since, older needles in older trees (> 2 years old) make up a larger proportion of foliage than in seedlings, and these older needles are much more severely affected by SNC than younger needles are, it is not surprising that the genetic correlation between the two traits were lower in older trees. In this study, all needles were less than 3-year-old at the assessment date and 1-year-old needles, on which the needles retention was scored, made up one third of the foliage. As a result there is a high genetic correlation between needle retention and foliage density in younger trees.

4.4.2 Geographic Variation

Since the two color traits and the two retention traits are controlled virtually by the same sets of genes, as evidenced by near unity genetic correlations (Table 4.6), geographic variation was investigated only in foliage color and foliage density

because of they are slightly more heritable than needle color and needle retention (Table 4.7).

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%), however, could be explained by a significant (best fitting) model containing a subset of the geography and climate variables (Table 4.8).

Table 4.8. Regression equation for foliage color.

Variable	Parameter Estimate	Partial R^2	P-value
Intercept	1.8829	--	<0.0001
Distance to the Pacific Ocean	0.0179	0.0296	0.0053
(Distance to the Pacific Ocean)^2	$-5.0163 * 10^{-10}$	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)^2	$-5.1643 * 10^{-7}$	0.0332	0.0032
	Model (Cp = 5.72):	0.1446	0.0003

While this model only explains 14% of the total variation in foliage color it gives some clues about factors that might be influencing tolerance of Douglas-fir to SNC. According to the model, color score improves (i.e., families are more tolerant to SNC, at least as expressed by foliage color) with increasing distance of mother trees from the Pacific Ocean up to a point (approximately 20 km from the

ocean), and then scores decrease with increasing distance. This is probably because moisture and temperature conditions are probably optimum for *P. gaeumannii* infection and SNC symptom development at this distance from the ocean. Areas that are closer to the ocean are probably too cool and moist, and areas more inland are too warm and dry for SNC symptom development and, thus, there probably has not been sufficient pressure by SNC for the selection of tolerant Douglas-fir genotypes in these areas.

Seed sources from southerly aspects (cosine(aspect) < 0) were found to have greener foliage than the sources facing north. In the northern hemisphere, exposure to sun is greater in the southerly aspects. The environmental conditions in such areas are harsher than northerly aspects in terms of temperature and moisture. These harsh conditions probably increase the severity of SNC symptoms and only tolerant Douglas-fir genotypes could occupy such areas in the presence of SNC. The effect of aspect, however, seems to decrease with increasing latitude (i.e., further north) of mother tree locations as indicated by the significant interaction between the two variables.

The higher the elevation of mother tree locations, the lower the foliage color scores according to this model. Temperature and moisture conditions in the higher elevations are probably not suitable for SNC and thus, Douglas-fir genotypes originating from such areas are more susceptible to the disease.

Thus, 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 by SNC.

Relationships between disease tolerance and geographical variables have also been reported in other conifer species. In two separate studies, Rehfeldt (1987) and Hunt *et al.* (1987) reported increased resistance to *Lophodermella* needle cast (*Lophodermella concolor* [Dearn.] Darker) in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) from lower elevations and maritime areas.

Similar results were obtained for *Rhabdocline* needle cast (*Rhabdocline pseudotsugae* Syd.) of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) (Hoff 1987) and for *Meria* needle cast (*Meria laricis* Vuill.) of western larch (*Larix occidentalis* Nutt.) (Rehfeldt 1995). Northern provenances were found to be more resistant to *Lophodermium* needle cast (*Lophodermium seditiosum* Minter, Staley & Millar) in both Scots pine (*Pinus sylvestris* L.) (Stephan and Krusche 1986) and ponderosa pine (*Pinus ponderosa* Laws.) (caused by *Lophodermium baculiferum* Mayr) (Hoff 1988).

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 that SNC has not been applying significant selection pressure in the past.

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Chapter 5

Summary and Conclusions

This research focused on the quantitative genetics and early testing of Swiss needle cast (SNC) tolerance in Douglas-fir, and geographic variation in Douglas-fir with respect to SNC tolerance.

In Chapter 2, the relationship between *P. gaeumannii* infection and SNC symptom severity was investigated using 6 Douglas-fir families varying in SNC symptom severity. Severity of SNC symptoms and amount of *P. gaeumannii* infection were compared. The main findings from this study were:

- Amount of *P. gaeumannii* infection did not differ significantly among Douglas-fir families, even between the most and the least tolerant families. Thus, variation in severity of SNC symptoms in Douglas-fir appears to be associated with tolerance, not resistance.
- Visual assessment of foliage traits (i.e., needle retention, needle color) related to SNC tolerance in the field is as reliable as more time consuming and detailed laboratory assessments of the same traits.
- Average retention of needles produced in the past four growing seasons can be used to assess tolerance.

Chapter 3 dealt with genetics and early testing of Douglas-fir for SNC tolerance. Two-year-old and 10- and 12-year-old progeny of 55 wind-pollinated Douglas-fir families were evaluated both in the field and in the laboratory to investigate the possibility of early selection for SNC tolerance.

- Natural inoculation of the subject seedlings from *P. gaeumannii* spores released from the surrounding infected Douglas-fir stands was found to be sufficient for symptom development. Seedlings can also be artificially infected by suspending infected Douglas-fir branches over

them in a growth chamber, but environmental conditions must be carefully controlled during and after inoculations for symptom development.

- Field assessment of foliage traits seemed sufficient for early testing purposes. None of the traits assessed in the laboratory were sufficiently superior to visually assessed field traits to justify replacing field assessments.
- Foliage traits were under low to moderate genetic control and controlled largely by the same sets of genes in the seedlings and older trees. Thus, it is possible to breed Douglas-fir for increased tolerance to SNC.
- Early testing is possible for needle retention and color due to the high correlation between the two ages for these traits.
- Early testing for SNC tolerance can be incorporated into current Douglas-fir breeding in two ways. First, new families to be progeny tested can be screened for SNC tolerance in seedlings and poor performing families eliminated before filed 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.
- There were no statistically significant differences among the 55 families in terms of amount of fungal infection. This finding supports the conclusion in Chapter 2 that variation in SNC symptom severity is associated with tolerance.

In Chapter, 4 SNC tolerance in Douglas-fir with respect to geographic and climatic variation was investigated in the Siuslaw National Forest. Two-year-old progeny of 152 open-pollinated Douglas-fir families were visually assessed for needle retention, foliage density, needle color and foliage color in two seedling trials and severity of SNC symptoms were compared to geography and climatic variables of mother tree locations. The main findings were:

- Similar to Chapter 3 results, needle and foliage traits were under weak genetic control and under the control of largely overlapping sets of genes.
- No relationship was found between location variables and foliage retention, but there was some evidence that foliage color is associated with elevation, distance to the Pacific Ocean, aspect and latitude of mother tree location ($R^2 = 0.14$). Families from lower elevations with southerly aspects had greener foliage, but the effect of aspect decreases with increased latitude. Color improved with increased distance from the Pacific Ocean up to about 20 km and then decreased as distance from the ocean increased further.
- It appears that color is more responsive to selection by SNC, but overall, SNC probably has not applied sufficient selection pressure to cause large geographic differentiation in Douglas-fir with respect to SNC tolerance.

Longevity of foliage is often associated with production and maintenance cost of 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, foliage is retained longer. It has been shown that photosynthetic rate of Douglas-fir needles decreases when infected by *P. gaeumannii* (Manter *et al.* 2000). In Chapter 2, it is reported that infected needles are shed sooner in tolerant families than in less tolerant families for a given amount of infection. If, in fact, the reason for the difference between more tolerant and less tolerant families is the cost-benefit relationship differences in their foliage, determining these differences would be a useful tool for determining tolerant Douglas-fir families in the future. This, cost-benefit hypothesis for SNC tolerance needs further investigation.

In Chapter 3, the seedlings were naturally inoculated with *P. gaeumannii* spores released from surrounding Douglas-fir stands at the trial sites. If needed,

seedlings can also be inoculated artificially. Artificial inoculation by suspending infected Douglas-fir branches over subject seedlings in a growth chamber may be an alternative inoculation technique for inoculating large number of seedlings. While the latter method is frequently and effectively used in artificially inoculating smaller number of seedlings (Capitano 1999, SNCC Annual Reports 1998-2001, Oregon State University, Corvallis, Oregon), its efficiency in inoculating larger number of seedlings, which is usually the case in breeding programs, needs to be tested. Optimum conditions for infection and symptom development should be determined.

The relationship between SNC symptom severity, at least for foliage color, and geography and climatic conditions of mother tree locations was significant but very weak. This is in contrast to the strong associations found between variation in growth and phenology traits and geography of parent tree locations in other studies of coastal Douglas-fir (e.g. Campbell and Sorensen 1978, Campbell 1979, Sorensen 1983). One reason for the poor relationship found with SNC might be the relatively small area investigated in this study. Limited evidence presented in Chapter 4 suggests that a portion of variation in color is associated with geography, thus, suggesting that relationships in a larger area may yield more precise information on how SNC tolerance varies across coastal Oregon.

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Appendices

Appendix A

This appendix includes a table listing family means for each variable, and two figures that show relationship between amount of fungal DNA and needle retention at individual sites reported in chapter 2.

Appendix Table A1. Variable means for each family in three disease severity groups.

OVER BOTH SITES

	DISEASE SEVERITY GROUPS											
	LIGHT				MODERATE				SEVERE			
Family No:	3906		3722		3493		3739		3459		3577	
Field Traits												
FNR*	5.13		4.63		3.97		3.99		4.13		3.63	
FNC	1.65		1.60		1.57		1.50		1.46		1.26	
FFC	2.78		2.78		2.28		2.22		2.33		1.83	
FD	4.50		4.28		3.33		3.28		3.00		2.56	
DBH	178.72		178.78		162.89		155.72		141.28		129.78	
BRD	22.26		19.28		20.16		18.13		17.50		16.24	
Lab. Traits	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998
LNR	80.53	69.94	76.46	63.68	76.21	54.57	81.53	59.91	75.61	55.03	74.62	54.76
LNC	3.89	3.31	3.39	3.11	3.53	2.81	3.33	3.14	3.53	2.64	3.22	2.36
PSP	2.28	9.48	2.98	8.88	3.39	10.96	3.16	9.88	2.97	8.79	3.98	9.42
DNA	65.70	253.41	116.95	262.14	117.74	258.16	90.31	296.41	83.75	321.35	129.36	307.42
NL	296.11	302.19	253.81	270.14	264.29	274.66	246.18	251.43	268.15	275.88	260.39	271.14
FRW	0.65	0.79	0.55	0.63	0.56	0.65	0.48	0.56	0.53	0.63	0.48	0.55
DRW	0.34	0.40	0.30	0.34	0.28	0.35	0.24	0.28	0.27	0.33	0.24	0.28
IL	16.33	19.42	12.97	16.72	13.77	17.28	11.86	16.22	13.50	17.06	11.86	15.44
PNSA	28.31	25.05	28.92	26.76	28.51	25.28	30.82	26.75	29.92	28.02	29.01	27.39
MOIST	0.31	0.39	0.25	0.29	0.28	0.31	0.23	0.28	0.26	0.29	0.24	0.27

*FNR = Needle retention, FNC = Needle color, FFC = Foliage color, FD = Foliage density, DBH = Diameter at breast height, BRD = Branch diameter, LNR = Needle retention, LNC = Needle color, PSP = Proportion of stomata with pseudothecia, DNA = Amount of fungal DNA, NL = Needle length FRW = Needle fresh weight, DRW = Needle dry weight, IL = Internode length, PNSA = Projected needle specific area, MOSIT = Needle moisture.

Appendix Table A1 (Continued)

ACEY CREEK

	DISEASE SEVERITY GROUPS											
	LIGHT				MODERATE				SEVERE			
Family No:	3906		3722		3493		3739		3459		3577	
Field Traits												
FNR	4.94		4.42		3.64		3.53		4.03		3.75	
FNC	1.53		1.64		1.67		1.33		1.44		1.42	
FFC	2.78		2.89		2.22		2.22		2.33		1.89	
FD	4.56		4.67		3.33		3.44		3.22		2.78	
DBH	190.44		185.78		159.56		148.89		141.22		138.11	
BRD	22.31		20.64		21.30		16.19		17.72		16.94	
Lab. Traits	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998
LNR	82.74	68.59	74.87	63.47	76.23	49.96	78.55	58.86	72.28	59.62	71.17	52.97
LNC	3.78	3.39	3.28	3.22	3.28	3.22	3.33	3.17	3.50	2.94	3.39	3.11
PSP	2.49	11.37	3.43	9.47	3.88	12.23	3.63	10.62	2.86	8.76	5.87	11.82
DNA	59.57	305.03	139.27	273.44	114.26	301.66	99.69	254.18	67.52	318.77	185.98	359.25
NL	290.80	286.08	272.23	281.99	269.92	278.41	240.68	261.98	281.99	279.52	271.40	277.83
FRW	0.65	0.75	0.65	0.67	0.61	0.66	0.45	0.61	0.56	0.63	0.55	0.60
DRW	0.35	0.38	0.36	0.38	0.31	0.37	0.24	0.30	0.29	0.35	0.27	0.32
IL	16.08	19.36	14.43	16.98	16.39	19.28	11.20	16.98	14.78	18.01	13.88	16.79
PNSA	27.65	24.67	26.48	26.63	26.29	25.52	30.65	26.29	29.43	28.63	27.34	26.35
MOIST	0.31	0.37	0.28	0.29	0.30	0.29	0.22	0.31	0.27	0.29	0.28	0.28

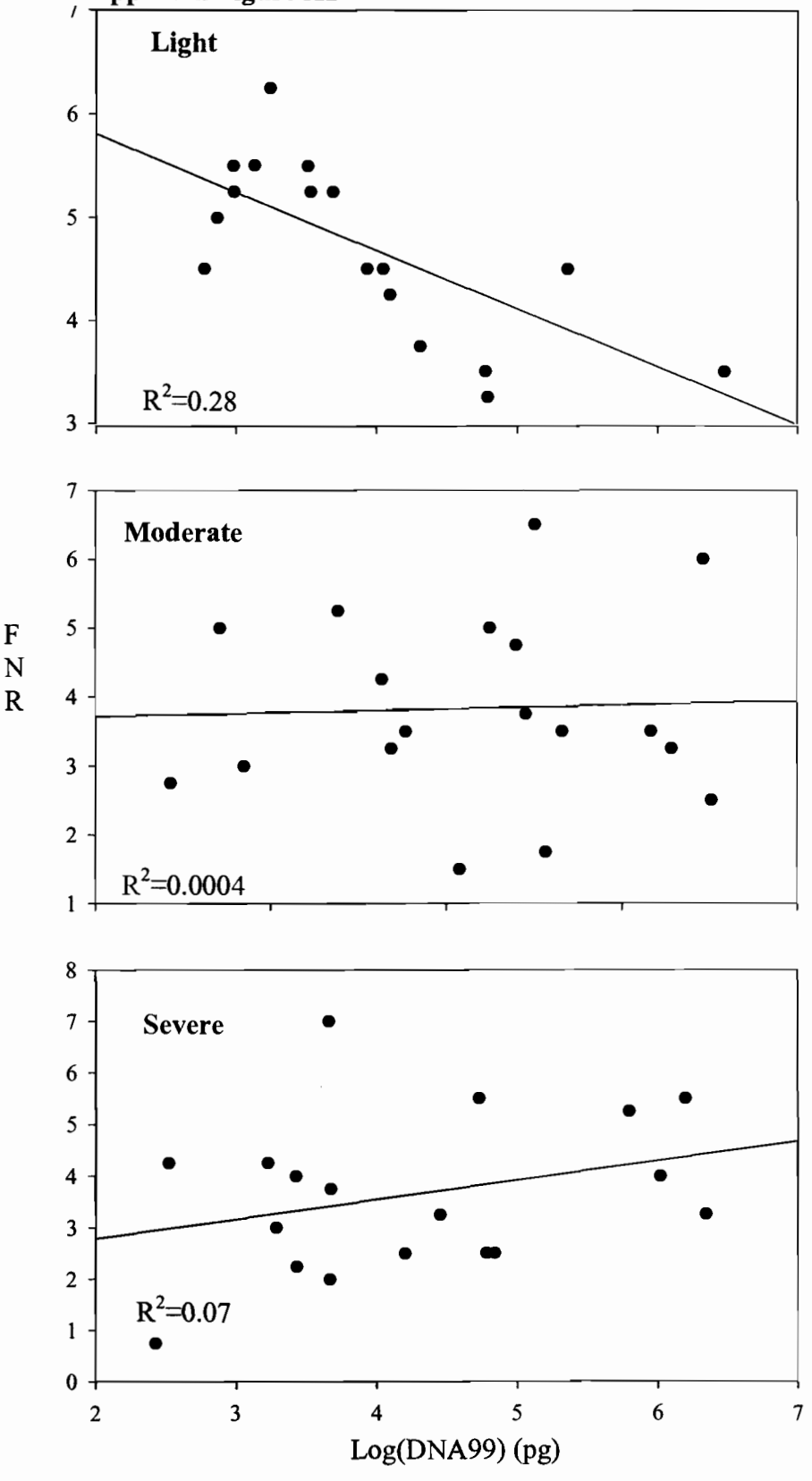
Appendix Table A1 (Continued)

COAL CREEK

	DISEASE SEVERITY GROUPS											
	LIGHT				MODERATE				SEVERE			
Family No:	3906		3722		3493		3739		3459		3577	
Field Traits												
FNR	5.31		4.83		4.31		4.44		4.22		3.50	
FNC	1.78		1.56		1.47		1.67		1.47		1.11	
FFC	2.78		2.67		2.33		2.22		2.33		1.78	
FD	4.44		3.89		3.33		3.11		2.78		2.33	
DBH	167.00		171.78		166.22		162.56		141.33		121.44	
BRD	22.21		17.92		19.02		20.07		17.28		15.53	
Lab. Traits	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998
LNR	78.32	71.29	78.05	63.88	76.20	59.18	84.52	60.95	78.94	50.44	78.08	56.56
LNC	4.00	3.22	3.50	3.00	3.78	2.39	3.33	3.11	3.56	2.33	3.05	1.61
PSP	2.07	7.59	2.52	8.29	2.91	9.69	2.68	9.14	3.08	8.82	2.09	7.01
DNA	71.84	201.79	94.63	250.83	121.22	214.66	80.93	338.63	99.97	323.94	72.75	255.60
NL	301.42	318.31	235.40	258.29	258.66	270.90	251.68	240.88	254.31	272.24	249.38	264.45
FRW	0.65	0.83	0.44	0.59	0.51	0.65	0.48	0.51	0.49	0.62	0.41	0.50
DRW	0.34	0.43	0.23	0.29	0.24	0.32	0.24	0.26	0.24	0.33	0.22	0.25
IL	16.57	19.47	11.51	16.45	11.14	15.28	12.52	15.46	12.21	16.12	9.84	14.08
PNSA	28.96	25.44	31.36	26.90	30.73	25.04	30.98	27.21	30.40	27.42	30.68	28.42
MOIST	0.31	0.40	0.22	0.29	0.27	0.32	0.25	0.25	0.25	0.29	0.19	0.25

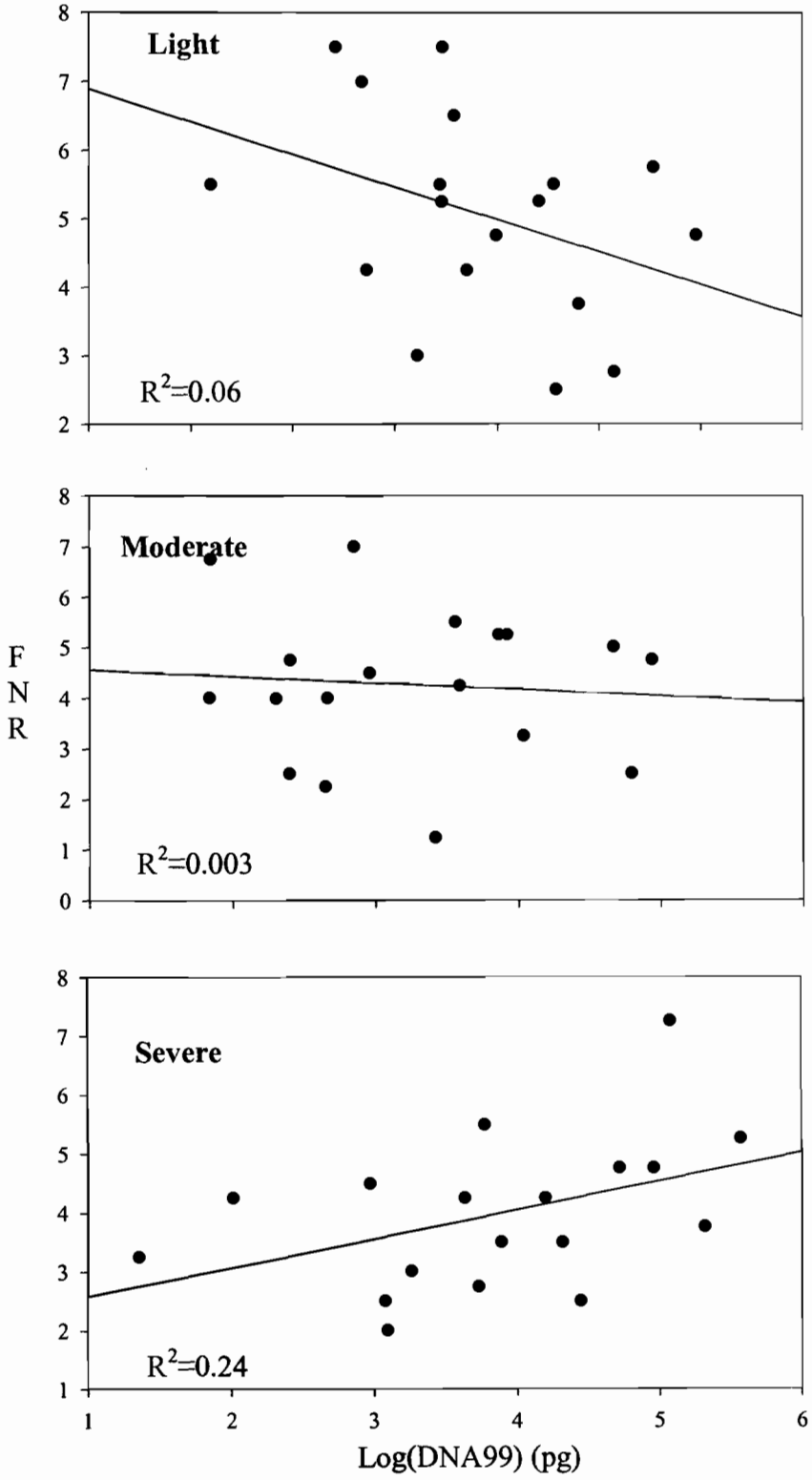
Appendix Figure A1. Relationship between log(fungal DNA content in 1999 needles) and field needle retention in Acey Creek.

Appendix Figure A1



Appendix Figure A2. Relationship between log(fungal DNA content in 1999 needles) and field needle retention in Coal Creek.

Appendix Figure A2



Appendix B

This appendix lists the data used in chapter 3. ANOVA tables for each variable and variograms for the rest of the replications are also included.

Appendix Table B1. Family means for mature test sites (FN = Family Number, FD = Foliage Density, FC= Foliage Color, NR = Needle Retention).

		GORDY			SALAL			BOTH SITES		
Order	FN	FD	FC	NR	FD	FC	NR	FD	FC	NR
1	60	3.6250	1.8438	7.6875	3.6250	1.8750	5.3750	3.6250	1.8542	6.9167
2	61	3.3684	2.0000	7.1579	3.6071	2.1071	7.1786	3.4697	2.0455	7.1667
3	62	4.2500	1.9722	8.3611	4.7692	2.4231	8.1154	4.4677	2.1613	8.2581
4	63	4.0313	1.9063	7.6250	3.9375	2.1875	7.5313	3.9844	2.0469	7.5781
5	64	3.9706	1.9706	7.5882	4.4091	2.1818	7.0000	4.1429	2.0536	7.3571
6	65	3.7000	1.8000	6.4667	3.0385	2.0769	6.1154	3.3929	1.9286	6.3036
7	66	3.7368	1.9737	7.6053	3.8571	1.9643	6.8929	3.7879	1.9697	7.3030
8	67	3.5294	1.8824	8.2647	3.3846	2.1539	6.2692	3.4667	2.0000	7.4000
9	68	3.7778	1.7778	7.4167	3.7222	1.9167	6.4444	3.7500	1.8472	6.9306
10	69	3.9375	1.9063	7.7813	3.8750	2.1667	6.9167	3.9107	2.0179	7.4107
11	70	4.1250	2.0000	7.9688	3.8636	2.3636	7.5909	4.0185	2.1482	7.8148
12	71	3.4375	1.6875	7.3750	3.4615	1.7692	5.7083	3.4483	1.7241	6.6607
13	72	3.9722	1.9722	7.1667	4.3750	2.3333	7.4167	4.1333	2.1167	7.2667
14	73	3.5790	1.8947	6.9474	3.2857	1.8214	6.1923	3.4546	1.8636	6.6406
15	74	4.0000	1.6667	6.9444	4.3636	2.0455	6.9546	4.1379	1.8103	6.9483
16	75	3.8438	1.9063	7.0625	4.2500	2.2857	7.0667	4.0333	2.0833	7.0645
17	76	3.8529	1.8529	7.0294	4.2500	2.2857	6.8571	4.0323	2.0484	6.9516
18	77	3.9375	2.0000	7.5000	3.8214	1.7857	5.8929	3.8833	1.9000	6.7500
19	78	3.7222	1.8056	8.0278	3.7500	2.1250	7.1250	3.7333	1.9333	7.6667
20	79	4.0588	1.9412	7.6471	4.2308	2.0000	6.4615	4.1333	1.9667	7.1333
21	80	3.5000	1.9211	7.2368	3.7308	2.0385	5.8333	3.5938	1.9688	6.6936
22	81	4.2000	1.8333	7.2000	4.5938	1.9412	5.8235	4.4032	1.8906	6.4688
23	82	3.8438	1.9063	7.6563	3.6875	1.8125	5.9688	3.7656	1.8594	6.8125
24	83	4.2105	2.0263	7.6842	3.8462	2.1539	6.3462	4.0625	2.0781	7.1406

Appendix Table B1 (Continued)

		Gordy			Salal			Both Sites		
Order	FN	FD	FC	NR	FD	FC	NR	FD	FC	NR
25	84	3.6389	1.6667	7.1944	3.4615	1.5385	5.5385	3.5645	1.6129	6.5000
26	85	3.4474	1.8158	7.4474	3.6111	1.8889	5.6111	3.5000	1.8393	6.8571
27	86	3.8889	2.2500	8.0556	4.4615	2.6539	6.9643	4.1290	2.4194	7.5781
28	87	3.8947	2.0790	8.1053	3.7500	1.7857	4.9615	3.8333	1.9546	6.8281
29	88	3.6579	1.9737	7.6842	3.8077	2.2308	8.1539	3.7188	2.0781	7.8750
30	89	3.5000	1.8824	7.5588	3.4286	2.0000	6.8929	3.4677	1.9355	7.2581
31	90	3.8889	1.9722	7.8056	3.7083	1.7083	6.4583	3.8167	1.8667	7.2667
32	91	3.7368	1.9474	7.4737	4.0833	2.0417	6.3333	3.8710	1.9839	7.0323
33	92	4.0556	1.7778	7.5556	4.0000	2.0333	6.4667	4.0303	1.8939	7.0606
34	93	3.7353	2.1177	8.2059	3.9286	2.0714	7.0714	3.7917	2.1042	7.8750
35	94	3.9706	2.0588	7.9706	3.8571	1.9286	6.6429	3.9194	2.0000	7.3710
36	95	4.0938	2.0313	8.1563	3.8333	2.0333	5.8333	3.9677	2.0323	7.0323
37	96	4.1667	2.2000	8.2667	3.3235	1.8824	6.4118	3.7188	2.0313	7.2813
38	97	3.9667	1.9000	7.6667	3.6250	1.9167	6.4583	3.8148	1.9074	7.1296
39	98	3.7353	1.9118	7.5588	3.5667	1.9000	6.4667	3.6563	1.9063	7.0469
40	99	3.8684	1.9474	8.0790	3.8333	1.8667	7.3333	3.8529	1.9118	7.7500
41	100	3.4167	1.9722	7.5833	3.8000	1.6667	5.2667	3.5909	1.8333	6.5303
42	101	3.4688	1.8750	7.6563	3.5000	1.8235	6.3235	3.4849	1.8485	6.9697
43	102	3.9412	1.9118	7.6177	3.7143	1.7500	5.6071	3.8387	1.8387	6.7097
44	103	3.7059	2.0882	8.2941	3.8929	2.5714	7.1429	3.7903	2.3065	7.7742
45	104	3.7059	2.0000	7.5294	3.8929	2.0357	6.5357	3.7903	2.0161	7.0807
46	105	3.8333	1.9167	7.2500	3.9000	1.9667	5.7000	3.8636	1.9394	6.5455
47	106	3.7368	1.9211	7.7895	3.4091	1.9091	7.5455	3.6167	1.9167	7.7000
48	107	3.6389	1.9167	7.4444	3.5625	1.9375	6.9688	3.6029	1.9265	7.2206
49	108	3.9706	2.0882	8.0588	4.0333	2.1333	7.8000	4.0000	2.1094	7.9375
50	109	3.7368	1.8611	8.2500	3.8750	1.9063	7.0938	3.8000	1.8824	7.7059

Appendix Table B1 (Continued)

		Gordy			Salal			Both Sites	
Order	FN	FD	FC	NR	FD	FC	NR	FD	FC	NR
51	110	3.5000	1.7632	7.7895	3.6667	2.0667	7.2667	3.5735	1.8971	7.5588
52	111	3.6667	1.8889	7.2500	3.5313	2.2188	7.1250	3.6029	2.0441	7.1912
53	112	3.7059	1.9706	7.8824	3.6818	1.9091	6.4546	3.6964	1.9464	7.3214
54	113	3.5938	2.0000	8.0000	3.7500	2.1500	7.1500	3.6539	2.0577	7.6731
55	114	4.3438	1.8750	7.9375	4.5000	2.1429	6.8571	4.4167	2.0000	7.4333

Appendix Table B2a. Family means for needle color (NC), needle retention (NR) Foliage density (FD) and foliage color (FC) assessed in fall 2000 at the juvenile test sites (family means for summer assessments are given in Appendix C).

		Both Sites				Pleasant Valley				Toledo			
Or	FAMILY	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
1	60	1.611	3.500	2.389	1.694	1.737	2.947	2.000	1.842	1.471	4.118	2.824	1.529
2	61	1.417	3.028	2.083	1.444	1.250	2.450	1.900	1.300	1.625	3.750	2.313	1.625
3	62	1.595	3.270	2.135	1.568	1.600	2.900	2.100	1.500	1.588	3.706	2.176	1.647
4	63	1.553	3.421	2.395	1.553	1.600	3.350	2.550	1.600	1.500	3.500	2.222	1.500
5	64	1.359	3.897	2.769	1.359	1.350	3.000	2.300	1.350	1.368	4.842	3.263	1.368
6	65	1.436	2.744	1.923	1.436	1.450	2.400	1.800	1.450	1.421	3.105	2.053	1.421
7	66	1.487	3.359	2.179	1.436	1.526	3.053	2.158	1.421	1.450	3.650	2.200	1.450
8	67	1.636	4.545	2.848	1.727	1.600	4.250	2.750	1.700	1.692	5.000	3.000	1.769
9	68	1.632	4.263	2.816	1.553	1.700	4.050	2.650	1.550	1.556	4.500	3.000	1.556
10	69	1.324	1.595	1.432	1.405	1.300	1.150	1.300	1.400	1.353	2.118	1.588	1.412
11	70	1.457	4.543	2.886	1.514	1.350	3.450	2.400	1.400	1.600	6.000	3.533	1.667
12	71	1.270	2.378	1.838	1.297	1.200	1.500	1.300	1.200	1.353	3.412	2.471	1.412
13	72	1.583	4.583	3.056	1.583	1.450	4.650	3.200	1.450	1.750	4.500	2.875	1.750
14	73	1.421	1.605	1.395	1.395	1.450	1.200	1.300	1.400	1.389	2.056	1.500	1.389
15	74	1.444	2.806	2.000	1.472	1.526	3.316	2.105	1.579	1.353	2.235	1.882	1.353
16	75	1.625	3.875	2.500	1.625	1.400	1.800	1.350	1.450	1.850	5.950	3.650	1.800
17	76	1.385	1.641	1.359	1.333	1.400	1.600	1.300	1.350	1.368	1.684	1.421	1.316
18	77	1.667	3.846	2.667	1.641	1.500	3.200	2.200	1.500	1.842	4.526	3.158	1.789
19	78	1.684	4.842	2.816	1.711	1.500	4.800	2.650	1.550	1.889	4.889	3.000	1.889
20	79	1.821	4.205	2.795	1.795	1.950	4.000	2.750	1.900	1.684	4.421	2.842	1.684
21	80	1.667	3.205	1.923	1.692	1.750	3.900	2.250	1.750	1.579	2.474	1.579	1.632
22	81	1.486	4.054	2.514	1.514	1.250	3.550	2.200	1.300	1.765	4.647	2.882	1.765
23	82	1.676	6.059	3.618	1.706	1.647	6.118	3.529	1.706	1.706	6.000	3.706	1.706
24	83	1.622	3.595	2.595	1.703	1.550	4.200	2.950	1.650	1.706	2.882	2.176	1.765
25	84	1.429	3.000	1.971	1.429	1.350	3.000	1.800	1.350	1.533	3.000	2.200	1.533
26	85	1.378	4.000	2.649	1.514	1.300	4.600	2.850	1.400	1.471	3.294	2.412	1.647
27	86	1.743	6.029	3.743	1.771	1.750	4.900	3.350	1.850	1.733	7.533	4.267	1.667
28	87	1.615	3.872	2.615	1.667	1.450	2.300	1.700	1.500	1.789	5.526	3.579	1.842
29	88	1.686	4.514	3.086	1.714	1.700	5.300	3.600	1.750	1.667	3.467	2.400	1.667
30	89	1.571	3.086	2.114	1.571	1.550	2.350	1.900	1.550	1.600	4.067	2.400	1.600
31	90	1.462	3.436	2.359	1.487	1.700	4.000	2.750	1.700	1.211	2.842	1.947	1.263
32	91	1.487	4.026	2.667	1.538	1.500	2.300	1.950	1.550	1.474	5.842	3.421	1.526
33	92	1.579	2.605	1.895	1.632	1.550	2.400	1.550	1.650	1.611	2.833	2.278	1.611
34	93	1.811	4.054	2.432	1.811	1.750	3.800	2.300	1.800	1.882	4.353	2.588	1.824
35	94	1.568	3.757	2.486	1.568	1.450	3.600	2.400	1.400	1.706	3.941	2.588	1.765
36	95	1.800	3.625	2.450	1.750	1.950	3.650	2.500	1.850	1.650	3.600	2.400	1.650
37	96	1.583	4.167	2.917	1.639	1.737	4.000	2.526	1.737	1.412	4.353	3.353	1.529
38	97	1.359	2.128	1.821	1.359	1.400	1.950	1.700	1.400	1.316	2.316	1.947	1.316

Appendix Table B2a (Continued)

		Both Sites				Pleasant Valley				Toledo			
Or	FAMILY	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
39	98	1.684	4.211	2.500	1.737	1.800	4.050	2.350	1.900	1.556	4.389	2.667	1.556
40	99	1.579	4.000	2.658	1.658	1.579	2.947	2.211	1.737	1.579	5.053	3.105	1.579
41	100	1.525	3.625	2.475	1.550	1.550	3.500	2.500	1.650	1.500	3.750	2.450	1.450
42	101	1.923	5.821	3.436	1.897	1.750	5.100	2.950	1.750	2.105	6.579	3.947	2.053
43	102	1.447	3.158	2.184	1.526	1.684	3.947	2.632	1.789	1.211	2.368	1.737	1.263
44	103	1.526	3.211	2.237	1.526	1.400	2.900	2.200	1.400	1.667	3.556	2.278	1.667
45	104	1.667	2.750	2.194	1.667	1.526	1.632	1.632	1.526	1.824	4.000	2.824	1.824
46	105	1.550	1.925	1.700	1.575	1.450	0.800	1.050	1.450	1.650	3.050	2.350	1.700
47	106	1.417	4.167	2.611	1.444	1.421	3.947	2.368	1.474	1.412	4.412	2.882	1.412
48	107	1.649	4.757	2.973	1.703	1.750	4.700	3.000	1.800	1.529	4.824	2.941	1.588
49	108	1.553	2.474	1.763	1.605	1.600	1.700	1.400	1.650	1.500	3.333	2.167	1.556
50	109	1.606	4.576	2.909	1.576	1.500	4.875	2.813	1.438	1.706	4.294	3.000	1.706
51	110	1.447	2.526	1.763	1.579	1.400	1.800	1.350	1.450	1.500	3.333	2.222	1.722
52	111	1.543	3.114	2.200	1.629	1.632	3.368	2.158	1.684	1.438	2.813	2.250	1.563
53	112	1.541	4.514	2.676	1.649	1.474	6.158	3.368	1.632	1.611	2.778	1.944	1.667
54	113	1.639	3.972	2.472	1.639	1.700	3.750	2.350	1.700	1.563	4.250	2.625	1.563
55	114	1.316	2.974	2.184	1.368	1.350	2.850	2.150	1.400	1.278	3.111	2.222	1.333

Appendix Table B2b. Juvenile traits assessed in the laboratory (NC = needle color, NR = needle retention, DRYWGT = dry weight, NL = needle length, PSEUDO = percent stomata occluded with pseudothecia, SPAREA = needle specific area, DNA = fungal DNA).

Pleasant Valley

Order	FN	NC	NR	DRYWGT	NL	PSEUDO	SPAREA	DNA
1	60	2.47	7.37	0.11	16.82	12.53	33.03	473.29
2	61	2.70	7.65	0.13	19.42	18.25	37.21	409.43
3	62	2.45	7.10	0.12	19.11	12.03	37.71	664.88
4	63	2.65	6.45	0.12	17.90	16.13	37.38	421.18
5	64	2.40	6.70	0.13	18.95	15.73	39.16	370.28
6	65	2.37	6.79	0.12	17.77	11.50	36.91	301.95
7	66	2.53	6.89	0.17	20.82	14.37	36.18	357.94
8	67	2.60	7.30	0.15	16.99	14.63	32.81	455.56
9	68	2.80	7.80	0.19	21.54	14.80	33.22	447.96
10	69	2.55	7.65	0.15	20.50	14.90	37.66	615.96
11	70	2.55	7.75	0.11	16.97	17.13	38.16	438.62
12	71	2.65	7.30	0.13	18.83	18.53	39.08	877.88
13	72	2.75	7.70	0.12	16.77	18.60	36.45	730.33

Appendix Table B2b (Continued)

Order	FN	NC	NR	DRYWGT	NL	PSEUDO	SPAREA	DNA
14	73	2.60	6.20	0.12	18.32	18.95	35.99	577.47
15	74	2.16	7.05	0.13	18.51	17.39	39.07	588.71
16	75	2.35	6.75	0.13	18.82	14.00	40.24	714.12
17	76	2.30	7.40	0.13	18.81	16.70	39.04	567.96
18	77	2.00	7.11	0.13	18.64	14.87	37.42	417.72
19	78	2.65	7.50	0.17	22.45	13.88	34.61	506.19
20	79	2.95	7.50	0.15	19.49	11.15	33.01	504.94
21	80	2.85	7.65	0.16	19.02	12.73	32.56	540.06
22	81	2.50	7.20	0.11	18.11	20.55	38.04	431.96
23	82	2.71	7.29	0.16	21.13	20.15	36.56	812.50
24	83	2.75	7.70	0.13	20.71	17.68	38.48	677.02
25	84	2.75	6.85	0.14	19.71	13.80	34.65	476.02
26	85	2.65	6.05	0.12	18.73	16.03	40.33	473.93
27	86	3.15	7.85	0.11	18.03	17.88	38.33	517.31
28	87	2.65	7.10	0.16	20.09	14.40	33.85	592.64
29	88	2.85	7.80	0.13	18.79	18.38	46.35	532.90
30	89	2.75	8.05	0.13	18.09	14.55	36.79	724.57
31	90	2.50	7.40	0.14	18.98	11.03	36.51	531.36
32	91	2.45	7.35	0.13	17.53	15.45	37.29	721.75
33	92	2.50	6.65	0.12	18.59	19.43	36.13	658.16
34	93	2.80	7.30	0.17	20.16	14.63	31.95	573.43
35	94	2.90	7.15	0.14	18.89	16.78	37.13	504.74
36	95	2.45	7.40	0.12	16.11	12.10	37.63	445.39
37	96	2.42	7.58	0.14	19.27	17.21	37.18	776.45
38	97	2.50	5.80	0.12	17.95	10.30	38.01	503.69
39	98	2.90	7.75	0.15	20.27	14.83	33.00	622.82
40	99	2.68	7.37	0.14	18.04	15.76	34.81	645.42
41	100	2.75	7.35	0.15	19.12	12.03	37.19	508.59
42	101	2.75	7.20	0.14	19.23	16.95	38.09	540.83
43	102	2.47	6.21	0.13	20.43	12.32	40.16	491.25
44	103	3.00	7.15	0.14	18.62	15.18	33.31	690.56
45	104	2.63	7.21	0.11	15.46	14.61	37.32	437.19
46	105	2.39	4.44	0.11	15.23	9.84	36.28	317.53
47	106	2.42	7.47	0.17	23.11	15.68	35.37	998.62
48	107	2.60	6.15	0.14	18.45	17.05	38.25	613.13
49	108	2.45	7.10	0.13	18.03	15.08	33.79	619.96
50	109	2.67	8.00	0.12	16.88	20.60	37.74	515.86
51	110	2.60	7.70	0.14	19.32	11.13	35.24	589.48
52	111	2.79	7.32	0.11	16.55	16.16	36.48	722.62
53	112	2.47	7.79	0.12	18.16	19.50	39.72	693.54
54	113	2.50	6.95	0.11	18.00	12.08	40.40	601.30
55	114	2.50	7.20	0.14	18.63	17.63	36.26	866.22

Appendix Table B2b (Continued)

Toledo

Order	FN	NC	NR	DRYWGT	NL	PSEUDO	SPAREA	DNA
1	60	2.71	8.00	0.13	17.11	21.24	33.66	729.68
2	61	2.82	7.29	0.14	20.02	20.62	40.16	787.91
3	62	2.53	7.37	0.14	18.66	13.87	34.32	787.33
4	63	2.50	7.56	0.17	22.55	13.50	36.57	1019.94
5	64	2.80	8.00	0.13	18.39	18.53	35.42	587.21
6	65	2.68	6.68	0.15	21.57	16.61	34.95	933.45
7	66	2.55	7.35	0.18	21.13	19.78	30.34	891.03
8	67	2.77	6.54	0.15	19.43	14.04	34.31	868.56
9	68	2.50	7.44	0.15	20.74	14.89	36.71	826.43
10	69	2.65	7.06	0.16	21.25	17.09	36.52	628.92
11	70	2.65	7.65	0.11	17.64	19.59	36.91	668.33
12	71	2.61	7.67	0.16	21.16	15.11	33.90	707.43
13	72	2.82	7.94	0.14	18.55	14.56	33.45	821.35
14	73	2.47	7.68	0.15	19.95	15.42	34.26	899.64
15	74	2.69	6.75	0.16	19.94	15.91	36.25	747.66
16	75	2.65	7.55	0.20	21.87	8.78	29.88	785.47
17	76	2.53	7.58	0.17	21.65	14.37	34.78	844.12
18	77	2.74	7.42	0.13	21.13	19.50	36.80	832.39
19	78	3.00	8.11	0.16	20.79	12.86	33.31	556.36
20	79	2.84	7.63	0.17	21.09	10.89	29.56	539.16
21	80	2.84	7.68	0.17	20.32	13.45	32.50	791.82
22	81	2.83	7.61	0.13	19.72	24.69	35.70	804.50
23	82	2.94	7.61	0.13	20.12	20.19	33.82	494.94
24	83	2.94	8.00	0.12	18.62	23.75	38.10	822.48
25	84	2.73	8.20	0.15	22.54	16.03	33.86	645.07
26	85	2.41	7.41	0.17	21.00	17.32	37.15	679.61
27	86	2.82	8.47	0.10	19.00	21.21	44.44	626.86
28	87	2.63	7.89	0.13	17.44	20.39	33.45	805.93
29	88	2.94	7.47	0.13	19.14	28.26	34.99	982.87
30	89	2.53	6.87	0.13	18.89	25.07	37.93	766.59
31	90	2.32	7.11	0.14	18.97	16.11	36.96	631.10
32	91	2.78	8.11	0.13	19.16	13.53	32.79	667.50
33	92	3.00	7.63	0.14	20.38	18.13	34.06	767.87
34	93	3.06	7.94	0.20	21.88	15.83	29.03	651.76
35	94	2.71	7.65	0.12	17.78	21.62	35.63	628.67
36	95	2.40	7.00	0.15	20.38	16.05	35.72	749.86
37	96	2.59	7.53	0.15	22.65	21.76	35.05	947.71
38	97	2.79	7.26	0.18	21.82	14.61	34.16	900.00
39	98	2.95	8.32	0.12	18.12	23.74	35.06	686.01
40	99	2.89	8.00	0.16	19.60	18.92	31.60	844.66

Appendix Table B2b (Continued)

Order	FN	NC	NR	DRYWGT	NL	PSEUDO	SPAREA	DNA
41	100	2.84	7.42	0.20	23.10	18.00	28.66	771.36
42	101	3.25	8.05	0.14	21.14	20.38	34.94	1115.49
43	102	2.65	6.75	0.16	20.94	14.00	31.98	653.66
44	103	2.78	7.44	0.12	17.02	20.72	33.90	579.86
45	104	2.82	7.29	0.15	18.45	9.56	29.91	557.58
46	105	2.80	6.45	0.13	17.74	13.13	34.76	430.16
47	106	2.87	7.13	0.12	21.05	14.87	35.11	673.98
48	107	2.72	6.83	0.18	21.91	14.61	34.16	883.01
49	108	2.72	7.78	0.13	19.51	16.81	31.81	865.87
50	109	3.12	7.88	0.14	20.86	14.74	32.15	766.58
51	110	3.15	7.65	0.19	23.33	15.25	33.79	959.05
52	111	2.69	7.75	0.15	19.85	14.59	32.04	693.17
53	112	2.61	7.72	0.14	19.91	22.22	37.35	660.72
54	113	2.94	7.65	0.13	18.44	22.41	35.40	715.92
55	114	2.79	7.26	0.16	21.32	20.26	32.84	827.97

Both Sites

Order	FN	NC	NR	DRYWGT	NL	PSEUDO	SPAREA	DNA
1	60	2.58	7.67	0.12	16.96	16.64	33.32	601.49
2	61	2.76	7.49	0.13	19.69	19.34	38.57	598.67
3	62	2.49	7.23	0.13	18.89	12.92	36.06	726.10
4	63	2.58	6.97	0.15	20.10	14.88	36.99	720.56
5	64	2.60	7.35	0.13	18.67	17.13	37.29	478.74
6	65	2.53	6.74	0.14	19.67	14.05	35.93	617.70
7	66	2.54	7.13	0.18	20.98	17.14	33.18	624.49
8	67	2.67	7.00	0.15	17.95	14.39	33.40	662.06
9	68	2.66	7.63	0.17	21.16	14.84	34.87	637.20
10	69	2.59	7.38	0.16	20.84	15.91	37.14	622.44
11	70	2.59	7.70	0.11	17.27	18.26	37.59	553.47
12	71	2.63	7.47	0.14	19.94	16.91	36.63	792.65
13	72	2.78	7.81	0.13	17.58	16.74	35.07	775.84
14	73	2.54	6.92	0.13	19.12	17.23	35.15	738.56
15	74	2.40	6.91	0.14	19.17	16.71	37.78	668.18
16	75	2.50	7.15	0.16	20.35	11.39	35.06	749.80
17	76	2.41	7.49	0.15	20.19	15.56	36.97	706.04
18	77	2.37	7.26	0.13	19.89	17.18	37.11	625.06
19	78	2.82	7.79	0.16	21.66	13.39	33.99	531.28
20	79	2.90	7.56	0.16	20.27	11.03	31.33	522.05
21	80	2.85	7.67	0.16	19.65	13.08	32.53	665.94
22	81	2.66	7.39	0.12	18.87	22.51	36.93	618.23
23	82	2.83	7.46	0.15	20.61	20.17	35.15	653.72

Appendix Table B2b (Continued)

Order	FN	NC	NR	DRYWGT	NL	PSEUDO	SPAREA	DNA
24	83	2.84	7.84	0.12	19.72	20.55	38.30	749.75
25	84	2.74	7.43	0.15	20.92	14.76	34.31	560.55
26	85	2.54	6.68	0.14	19.77	16.62	38.87	576.77
27	86	3.00	8.14	0.10	18.47	19.41	41.14	572.09
28	87	2.64	7.49	0.14	18.80	17.32	33.66	699.29
29	88	2.89	7.65	0.13	18.95	22.92	41.13	757.89
30	89	2.66	7.54	0.13	18.44	19.06	37.28	745.58
31	90	2.41	7.26	0.14	18.98	13.50	36.73	581.23
32	91	2.61	7.71	0.13	18.30	14.54	35.16	694.63
33	92	2.74	7.13	0.13	19.46	18.79	35.12	713.01
34	93	2.92	7.61	0.18	20.98	15.20	30.57	612.60
35	94	2.81	7.38	0.13	18.38	19.00	36.44	566.71
36	95	2.43	7.20	0.13	18.25	14.08	36.67	597.62
37	96	2.50	7.56	0.15	20.86	19.36	36.17	862.08
38	97	2.64	6.51	0.15	19.84	12.40	36.14	701.84
39	98	2.92	8.03	0.13	19.22	19.17	34.00	654.41
40	99	2.79	7.68	0.15	18.82	17.34	33.21	745.04
41	100	2.79	7.38	0.17	21.06	14.94	33.03	639.97
42	101	3.00	7.63	0.14	20.19	18.66	36.52	828.16
43	102	2.56	6.49	0.14	20.69	13.18	35.96	572.45
44	103	2.89	7.29	0.13	17.86	17.80	33.59	635.21
45	104	2.72	7.25	0.13	16.87	12.22	33.82	497.38
46	105	2.61	5.50	0.12	16.62	11.67	35.48	373.85
47	106	2.62	7.32	0.15	22.20	15.32	35.25	836.30
48	107	2.66	6.47	0.16	20.09	15.89	36.31	748.07
49	108	2.58	7.42	0.13	18.73	15.89	32.85	742.92
50	109	2.91	7.94	0.13	18.99	17.48	34.77	641.22
51	110	2.88	7.68	0.17	21.32	13.19	34.51	774.26
52	111	2.74	7.51	0.13	18.06	15.44	34.45	707.90
53	112	2.54	7.76	0.13	19.01	20.82	38.57	677.13
54	113	2.70	7.27	0.12	18.20	16.82	38.10	658.61
55	114	2.64	7.23	0.15	19.94	18.91	34.59	847.10

Appendix Table B3. ANOVA tables for SNC traits at Pleasant Valley.

Needle Color (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	3.138503	1.046168	1.88	0.1352
Family	54	64.750972	1.199092	2.15	0.0001
Rep.*Family	162	90.204889	0.556820	1.16	0.0952
Error	864	413.000000	0.478009		
Total	1083	571.094365			

Foliage Color (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	4.039298	1.346433	2.62	0.0529
Family	54	55.275168	1.023614	1.99	0.0005
Rep.*Family	162	83.345936	0.514481	1.54	<0.0001
Error	864	287.750000	0.333044		
Total	1083	430.410402			

Needle Retention (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	181.443518	60.481172	8.47	<0.0001
Family	54	880.781110	16.310761	2.28	<0.0001
Rep.*Family	162	1157.035332	7.142194	1.85	<0.0001
Error	864	3335.450000	3.860475		
Total	1083	5554.709960			

Foliage Density (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	46.217734	15.405911	5.03	0.0023
Family	54	413.908682	7.664976	2.50	<0.0001
Rep.*Family	162	496.049631	3.062035	1.57	<0.0001
Error	864	1686.300000	1.951736		
Total	1083	2642.476047			

Needle Color (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	3.623323	1.207774	2.45	0.0656
Family	54	31.258199	0.578856	1.17	0.2225
Rep.*Family	162	79.920777	0.493338	1.58	<0.0001
Error	863	269.866667	0.312708		
Total	1082	384.668965			

Appendix Table B3 (Continued)

Foliage Color (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	3.393047	1.131016	2.23	0.0870
Family	54	32.708094	0.605705	1.19	0.2006
Rep.*Family	162	82.266970	0.507821	1.69	<0.0001
Error	863	259.116667	0.300251		
Total	1082	377.484778			

Needle Retention (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	229.132172	76.377391	4.22	0.0066
Family	54	1598.027448	29.593101	1.64	0.0098
Rep.*Family	162	2929.622751	18.084091	1.98	<0.0001
Error	863	7867.466667	9.116416		
Total	1082	12624.249040			

Foliage Density (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	84.553160	28.184387	6.36	0.0004
Family	54	406.099098	7.520354	1.70	0.0061
Rep.*Family	162	718.391667	4.434516	2.01	<0.0001
Error	863	1908.316667	2.211259		
Total	1082	3117.360592			

LABORATORY TRAITS

Needle Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	41.635744	13.878581	17.52	<0.0001
Family	54	46.748317	0.865710	1.09	0.3302
Rep.*Family	162	128.308421	0.792027	2.00	<0.0001
Error	858	339.350000	0.395513		
Total	1077	556.042482			

Appendix Table B3 (Continued)

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	219.061115	73.020372	23.67	<0.0001
Family	54	407.428290	7.544968	2.45	<0.0001
Rep.*Family	162	499.653873	3.084283	1.46	0.0005
Error	858	1814.950000	2.115326		
Total	1077	2941.093278			

Dry Weight

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	0.029136	0.009712	12.53	<0.0001
Family	54	0.076673	0.001420	1.83	0.0020
Error	162	0.125564	0.000775		
Total	219	0.231373			

Specific Needle Area

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	145.577190	48.525730	2.38	0.0713
Family	54	1391.660284	25.771487	1.27	0.1321
Error	162	3297.400520	20.354324		
Total	219	4834.637994			

Needle Length

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	1641.984560	547.328187	21.02	<0.0001
Family	54	2376.219785	44.004070	1.69	0.0064
Rep.*Family	162	4217.824703	26.035955	2.27	<0.0001
Error	856	9804.262630	11.453578		
Total	1075	18040.291680			

Pseudothecia

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	11931.353590	3977.117863	7.43	<0.0001
Family	54	30969.341180	573.506318	1.07	0.3638
Rep.*Family	162	86718.336200	535.298372	2.29	<0.0001
Error	856	200017.716700	233.665557		
Total	1075	329636.747700			

Appendix Table B3 (Continued)

DNA

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	17335522.75	5778507.58	93.06	<0.0001
Family	54	4453094.52	82464.71	1.33	0.0902
Error	162	10059792.88	62097.49		
Total	219	31848410.15			

Appendix Table B4. ANOVA tables for SNC traits at Toledo.

Needle Color (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	14.258601	4.752867	9.10	<0.0001
Family	54	53.435519	0.989547	1.89	0.0012
Rep.*Family	162	84.657117	0.522575	1.22	0.0451
Error	811	347.450000	0.428422		
Total	1030	499.801237			

Foliage Color (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	8.841895	2.947298	5.74	0.0009
Family	54	37.108553	0.687195	1.34	0.0841
Rep.*Family	162	83.144692	0.513238	1.54	<0.0001
Error	811	270.816667	0.333929		
Total	1030	399.911807			

Needle Retention (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	30.113486	10.037829	3.14	0.0271
Family	54	293.298849	5.431460	1.70	0.0061
Rep.*Family	162	518.533933	3.200827	1.25	0.0271
Error	811	2071.60000	2.554377		
Total	1030	2913.546268			

Appendix table B4 (Continued)

Foliage Density (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	24.243778	8.081259	2.88	0.0378
Family	54	205.420931	3.804091	1.35	0.0760
Rep.*Family	162	454.939075	2.808266	1.61	<0.0001
Error	811	1416.966667	1.747185		
Total	1030	2101.570450			

Needle Color (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	9.78213473	3.260712	8.48	<0.0001
Family	54	31.38603037	0.581223	1.51	0.0256
Rep.*Family	162	62.32009425	0.384692	1.39	0.0024
Error	749	207.0166667	0.276391		
Total	968	310.504926			

Foliage Color (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	12.015547	4.005182	10.67	<0.0001
Family	54	27.487829	0.509034	1.36	0.0752
Rep.*Family	162	60.803983	0.375333	1.37	0.0034
Error	749	204.600000	0.273164		
Total	968	304.907359			

Needle Retention (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	433.827332	144.609111	9.04	<0.0001
Family	54	1341.055863	24.834368	1.55	0.0188
Rep.*Family	162	2591.937334	15.999613	1.54	0.0001
Error	749	7783.583330	10.391967		
Total	968	12150.403860			

Foliage Density (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	90.335022	30.111674	7.46	0.0001
Family	54	356.278689	6.597754	1.63	0.0100
Rep.*Family	162	654.160595	4.038028	1.63	<0.0001
Error	749	1861.150000	2.484846		
Total	968	2961.924307			

Appendix table B4 (Continued)

LABORATORY TRAITS

Needle Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	40.278189	13.426063	17.89	<0.0001
Family	54	39.273469	0.727286	0.97	0.5410
Rep.*Family	162	121.567194	0.750415	2.15	<0.0001
Error	770	269.083333	0.349459		
Total	989	470.202185			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	31.732293	10.577431	4.95	0.0026
Family	54	193.813950	3.589147	1.68	0.0069
Rep.*Family	162	346.015326	2.135897	1.42	0.0015
Error	770	1161.850000	1.508896		
Total	989	1733.411569			

Dry Weight

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	0.057463	0.019154	12.31	<0.0001
Family	54	0.114634	0.002123	1.36	0.0714
Error	162	0.252112	0.001556		
Total	219	0.424209			

Specific Needle Area

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	1247.119493	415.706498	18.13	<0.0001
Family	54	1749.716239	32.402152	1.41	0.0514
Error	162	3714.612437	22.929706		
Total	219	6711.448169			

Needle Length

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	1011.119019	337.039673	9.96	<0.0001
Family	54	2337.384908	43.284906	1.28	0.01225
Rep.*Family	162	5483.392236	33.848100	2.10	<0.0001
Error	770	12437.176040	16.152177		
Total	989	21269.072200			

Appendix table B4 (Continued)

Pseudothecia

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	23857.495400	7952.498467	9.88	<0.0001
Family	54	59092.2213	1094.300394	1.36	0.0734
Rep.*Family	162	130360.009900	804.691419	2.09	<0.0001
Error	770	296677.633300	385.295628		
Total	989	509987.359900			

DNA

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	8275566.49	2758522.17	32.42	<0.0001
Family	54	4077014.77	75500.27	0.89	0.6893
Error	162	13783821.67	85085.32		
Total	219	26136402.93			

Appendix Table B5. ANOVA tables for SNC traits combined over both juvenile sites (55 families).

Needle Color (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	29.011628	29.011628	9.32	0.0189
Replication(Site)	6	17.397104	2.899517	5.37	<0.0001
Family	54	78.610583	1.455752	1.93	0.0084
Site*Family	54	40.631079	0.752427	1.39	0.0438
Rep*Fam.(Site)	324	174.862006	0.539698	1.19	0.0191
Error	1675	760.450000	0.454000		
Total	2114	1100.962399			

Foliage Color (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	38.251520	38.251520	17.32	0.0053
Replication(Site)	6	12.881192	2.146865	4.18	0.0004
Family	54	62.113666	1.150253	2.00	0.0060
Site*Family	54	31.049613	0.574993	1.12	0.2754
Rep*Fam.(Site)	324	166.490628	0.513860	1.54	<0.0001
Error	1675	558.566667	0.333473		
Total	2114	869.353286			

Appendix Table B5 (Continued)

Needle Retention (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	451.858008	451.858008	12.29	0.0111
Replication(Site)	6	211.557004	35.259501	6.82	<0.0001
Family	54	803.287006	14.875685	2.23	0.0019
Site*Family	54	360.222460	6.670786	1.29	0.0952
Rep*Fam.(Site)	324	1675.569264	5.171510	1.60	<0.0001
Error	1675	5407.050000	3.228090		
Total	2114	8909.543742			

Foliage Density (summer)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	127.929573	127.929573	10.77	0.0164
Replication(Site)	6	70.461511	11.743485	4.00	0.0007
Family	54	449.496167	8.324003	2.71	0.0002
Site*Family	54	165.594070	3.066557	1.04	0.3971
Rep*Fam.(Site)	324	950.988707	2.935150	1.58	<0.0001
Error	1675	3103.266667	1.852697		
Total	2114	4867.736695			

Needle Color (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	0.571529	0.571529	0.25	0.6313
Replication(Site)	6	13.405457	2.234243	5.09	<0.0001
Family	54	36.871067	0.682798	1.48	0.0767
Site*Family	54	24.925982	0.461592	1.05	0.3852
Rep*Fam.(Site)	324	142.240871	0.439015	1.48	<0.0001
Error	1612	476.883333	0.295833		
Total	2051	694.898238			

Foliage Color (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	0.465003	0.465003	0.18	0.6849
Replication(Site)	6	15.408595	2.568099	5.82	<0.0001
Family	54	34.222650	0.633753	1.39	0.1133
Site*Family	54	24.569237	0.454986	1.03	0.4232
Rep*Fam.(Site)	324	143.070953	0.441577	1.54	<0.0001
Error	1612	463.716667	0.287665		
Total	2051	681.453104			

Appendix Table B5 (Continued)

Needle Retention (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	186.046950	186.046950	1.67	0.2418
Replication(Site)	6	662.959504	110.493251	6.48	<0.0001
Family	54	1961.789412	36.329434	1.99	0.0063
Site*Family	54	985.197849	18.244405	1.07	0.3521
Rep*Fam.(Site)	324	5521.560085	17.041852	1.76	<0.0001
Error	1612	15651.050000	9.709088		
Total	2051	24968.603800			

Foliage Density (fall)

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	49.391268	49.391268	1.66	0.2415
Replication(Site)	6	174.888182	29.148030	6.88	<0.0001
Family	54	492.398331	9.118488	1.90	0.0099
Site*Family	54	258.855826	4.793626	1.13	0.2573
Rep*Fam.(Site)	324	1372.552263	4.236272	1.81	<0.0001
Error	1612	3769.466667	2.338379		
Total	2051	6117.552537			

LABORATORY TRAITS

Needle Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	10.988648	10.988648	0.82	0.4006
Replication(Site)	6	81.913933	13.652322	17.70	<0.0001
Family	54	55.709205	1.031652	1.91	0.0093
Site*Family	54	29.124001	0.539333	0.70	0.9447
Rep*Family(Site)	324	249.875615	0.771221	2.06	<0.0001
Error	1628	608.433333	0.373731		
Total	2067	1036.044736			

Appendix Table B5 (Continued)

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	67.308168	67.308168	1.58	0.2528
Replication(Site)	6	250.793408	41.798901	16.01	<0.0001
Family	54	421.956527	7.814010	2.29	0.0014
Site*Family	54	183.987956	3.407184	1.31	0.0853
Rep*Family(Site)	324	845.669199	2.610090	1.43	<0.0001
Error	1628	2976.800000	1.828501		
Total	2067	4746.515258			

Dry Weight

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	0.021700	0.021700	1.49	0.2676
Replication(Site)	6	0.086600	0.014433	12.38	<0.0001
Family	54	0.119119	0.002236	1.65	0.0342
Site*Family	54	0.072187	0.001337	1.15	0.2364
Error	324	0.377675	0.001166		
Total	439	0.677782			

Needle Specific Area

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	554.877045	554.877045	2.40	0.1729
Replication(Site)	6	1392.696684	232.116114	1.73	<0.0001
Family	54	2001.798075	37.070335	1.76	0.0203
Site*Family	54	1139.578449	21.103305	0.98	0.5283
Error	324	7012.012960	21.642015		
Total	439	12100.963210			

Needle Length

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	959.521160	959.521160	2.17	0.1915
Replication(Site)	6	2653.103579	442.183930	14.77	<0.0001
Family	54	3018.951054	55.906501	1.82	0.0152
Site*Family	54	1662.752187	30.791707	1.03	0.4079
Rep*Fam.(Site)	324	9701.216939	29.942028	2.19	0.4269
Error	1626	22241.438670	13.678622		
Total	2065	40236.983590			

Appendix Table B5 (Continued)

Proportion of Stomata Occluded With Pseudothecia

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	8315.783900	8315.783900	1.42	0.2789
Replication(Site)	6	35788.849000	5964.808167	8.90	<0.0001
Family	54	63129.983600	1169.073770	2.13	0.0031
Site*Family	54	29581.741000	547.810019	0.82	0.8021
Rep*Family(Site)	324	217078.346100	669.994895	2.19	0.8144
Error	1626	496695.350000	305.470695		
Total	2065	850590.053600			

DNA

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	3710520.68	3710520.68	0.87	0.3874
Replication(Site)	6	25611089.24	4268514.87	58.00	<0.0001
Family	54	4143775.69	76736.59	0.94	0.5824
Site*Family	54	4386333.59	81228.40	1.10	0.2983
Error	324	23843614.55	73591.40		
Total	439	61695333.75			

Appendix Table B6. ANOVA tables for mature traits at Salal.

Foliage Density

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Set	1	4.162089	4.162089	1.25	0.2971
Replication	4	20.208098	5.052025	2.10	0.2445
Replication*Set	4	9.603565	2.400891	2.43	0.0487
Family(Set)	53	108.626648	2.049559	2.06	0.0001
Rep.*Fam.(Set)	203	205.210745	1.010890	1.26	0.0248
Error	475	382.308417	0.804860		
Total	740	730.119562			

Foliage Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Set	1	3.492456	3.492456	0.76	0.4110
Replication	4	11.859973	2.964993	0.87	0.5503
Replication*Set	4	13.565842	3.391461	3.77	0.0054
Family(Set)	53	122.325306	2.308025	2.56	<0.0001
Rep.*Fam.(Set)	207	185.012063	0.893778	1.13	0.1485
Error	476	384.430893	0.807628		
Total	741	720.686533			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Set	1	2.113132	2.113132	1.08	0.3180
Replication	4	18.885563	4.721391	4.73	0.0808
Replication*Set	4	3.994078	0.998520	1.07	0.3723
Family(Set)	53	103.999600	1.962257	2.10	<0.0001
Rep.*Fam.(Set)	207	191.263180	0.923977	1.09	0.2298
Error	474	410.016616	0.865014		
Total	743	730.272169			

Appendix Table B7. ANOVA tables for mature traits at Gordy.

Foliage Density

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Set	1	0.518646	0.518646	0.07	0.8040
Replication	4	11.763379	2.940845	0.47	0.7588
Replication*Set	4	25.039602	6.259901	7.60	<0.0001
Family(Set)	53	127.069025	2.397529	2.91	<0.0001
Rep.*Fam.(Set)	212	173.992391	0.820719	0.92	0.7762
Error	679	608.159882	0.895670		
Total	953	946.542955			

Foliage Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Set	1	4.267420	4.267420	2.19	0.1731
Replication	4	10.524755	2.631189	2.15	0.2389
Replication*Set	4	4.904197	1.226049	1.21	0.3073
Family(Set)	53	92.591415	1.747008	1.72	0.0034
Rep.*Fam.(Set)	212	215.469023	1.016363	1.11	0.1679
Error	678	620.972144	0.915888		
Total	952	948.728954			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Set	1	16.614403	16.614403	3.46	0.0951
Replication	4	20.612855	5.153214	1.64	0.3211
Replication*Set	4	12.542423	3.135606	3.32	0.0114
Family(Set)	53	141.044344	2.661214	2.82	<0.0001
Rep.*Fam.(Set)	212	201.065017	0.948420	1.18	0.0663
Error	678	546.306379	0.805762		
Total	952				

Appendix Table B8. ANOVA tables for mature traits over Salal and Gordy.

Foliage Density

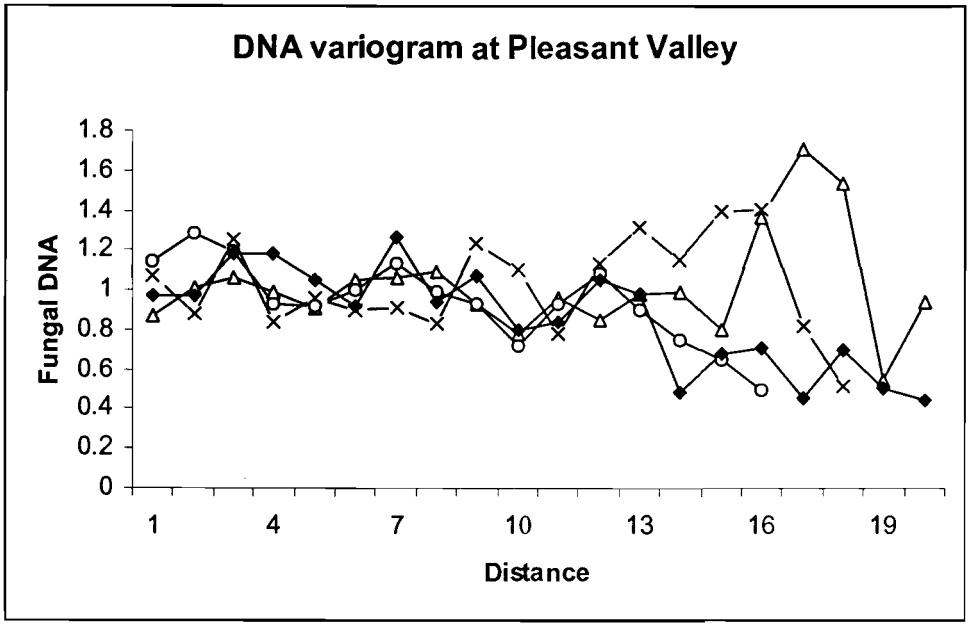
Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	0.499814	0.499814	0.55	0.8127
Replication(Site)	8	31.971478	3.996435	0.92	0.5434
Set	1	4.118852	4.118852	1.12	0.3213
Site*Set	1	1.230132	1.230132	0.30	0.6005
Set*Replication(Site)	8	34.643167	4.330396	4.76	<0.0001
Family(Set)	53	182.014409	3.434234	3.72	<0.0001
Site*Family(Set)	53	48.885268	0.922364	1.01	0.4491
Site*Rep.*Fam.(Set)	415	379.203137	0.913742	1.06	0.2149
Error	1154	990.468299	0.858291		
Total	1694	1673.034556			

Foliage Color

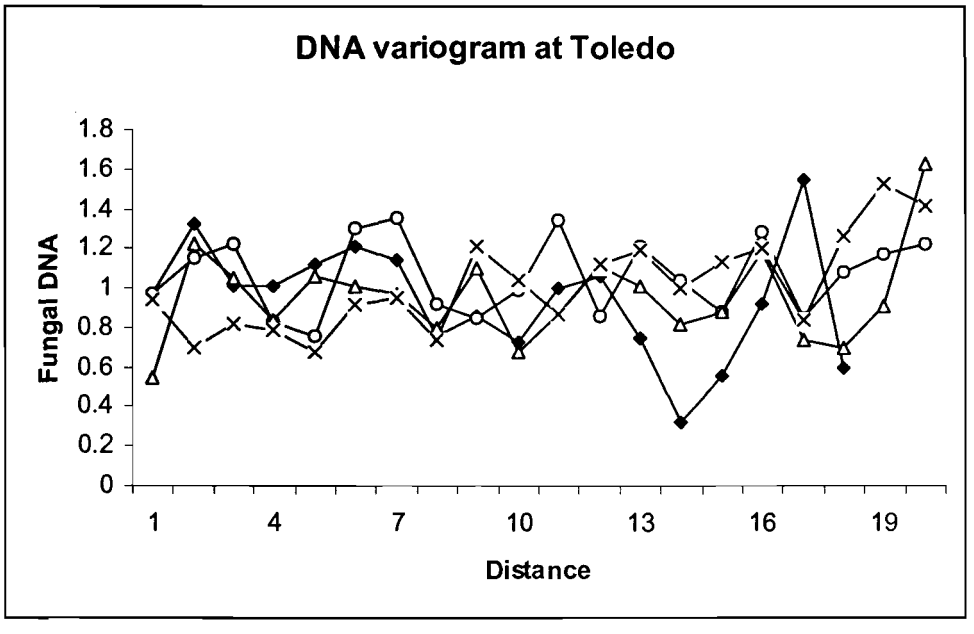
Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	0.019563	0.019563	0.01	0.9681
Replication(Site)	8	22.384729	2.798091	1.21	0.3962
Set	1	0.012381	0.012381	0.01	0.9749
Site*Set	1	7.573672	7.573672	2.91	0.1166
Set*Replication(Site)	8	18.470039	2.308755	2.41	0.0149
Family(Set)	53	144.657933	2.729395	2.03	0.0055
Site*Family(Set)	53	71.085996	1.341245	1.40	0.0374
Site*Rep.*Fam.(Set)	415	400.481086	0.965015	1.11	0.0994
Error	1154	1005.403038	0.871233		
Total	1694	1670.088437			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	0.000777	0.000777	0.01	0.9907
Replication(Site)	8	39.498418	4.937302	2.39	0.1198
Set	1	13.900335	13.900335	4.40	0.1691
Site*Set	1	2.173512	2.173512	0.78	0.3903
Set*Replication(Site)	8	16.536500	2.067063	2.20	0.0260
Family(Set)	53	145.386001	2.743132	1.58	0.0485
Site*Family(Set)	53	91.667612	1.729578	1.85	0.0005
Site*Rep.*Fam.(Set)	415	392.328197	0.945369	1.14	0.0512
Error	1152	956.322995	0.830141		
Total	1692	1657.814347			



Appendix Figure B1. Variograms of fungal DNA in each replication at Pleasant Valley.



Appendix Figure B2. Variograms of fungal DNA in each replication at Toledo.

Appendix C

This appendix includes data used in Chapter 4 and ANOVA tables for each variable.

Appendix Table C1. Family means for SCN traits over both sites and at each individual site (FN = family number; NC = needle color; FC = Foliage color, NR = needle retention; FD = foliage density).

		Both sites				Pleasant Valley				Toledo			
Order	FN	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
1	1	1.8056	7.3056	4.0556	1.7500	1.6316	6.6316	3.3684	1.5263	2.0000	8.0588	4.8235	2.0000
2	2	1.6857	7.3714	4.2286	1.6857	1.5263	6.3158	3.8947	1.5263	1.8750	8.6250	4.6250	1.8750
3	3	2.0270	7.9730	4.5135	2.0811	1.9500	7.8500	4.0500	1.9500	2.1177	8.1177	5.0588	2.2353
4	5	2.2162	7.8378	4.4865	2.3784	2.0526	6.9474	3.7895	2.1053	2.3889	8.7778	5.2222	2.6667
5	6	1.8462	7.9744	5.1026	1.9744	1.6842	7.8421	5.0000	1.7895	2.0000	8.1000	5.2000	2.1500
6	7	2.0270	7.6216	3.9730	2.0270	1.9000	7.2500	3.8000	1.9000	2.1765	8.0588	4.1765	2.1765
7	8	1.9143	8.0571	4.7143	2.0286	1.8333	7.3889	4.3889	1.7778	2.0000	8.7647	5.0588	2.2941
8	9	1.7879	7.3636	4.4546	1.8182	1.5000	7.1250	4.4375	1.5625	2.0588	7.5882	4.4706	2.0588
9	10	1.8462	8.5128	4.8205	2.0000	1.7500	8.1000	4.5000	1.7500	1.9474	8.9474	5.1579	2.2632
10	11	1.8919	6.7568	3.9189	1.9460	1.7368	5.5790	3.2105	1.6316	2.0556	8.0000	4.6667	2.2778
11	12	1.8750	7.8750	4.3000	2.0000	1.7500	7.2500	3.7500	2.0000	2.0000	8.5000	4.8500	2.0000
12	13	1.6316	7.7895	4.4737	1.7632	1.6000	7.6000	4.5000	1.7500	1.6667	8.0000	4.4444	1.7778
13	14	2.1250	7.9500	4.7750	2.0500	2.1500	7.5000	4.6000	2.1000	2.1000	8.4000	4.9500	2.0000
14	15	1.7000	8.0000	4.5500	1.9250	1.5000	7.5500	4.2000	1.5500	1.9000	8.4500	4.9000	2.3000
15	16	1.9444	8.6389	5.1944	2.0833	1.9000	8.5500	4.9500	2.0000	2.0000	8.7500	5.5000	2.1875
16	17	1.6410	7.9487	4.8974	1.8718	1.5000	7.9500	5.1500	1.8000	1.7895	7.9474	4.6316	1.9474
17	18	1.8500	6.9250	4.1000	1.8500	1.9000	6.4500	4.1500	1.9000	1.8000	7.4000	4.0500	1.8000
18	19	2.0263	7.9211	4.5263	2.0790	1.9000	7.3000	3.7000	1.9500	2.1667	8.6111	5.4444	2.2222
19	21	1.8000	7.7250	4.8750	1.9000	1.6000	7.0000	4.4500	1.7000	2.0000	8.4500	5.3000	2.1000
20	22	1.9474	8.3421	5.0263	1.9737	1.7500	7.8000	4.6500	1.7500	2.1667	8.9444	5.4444	2.2222
21	23	1.6410	7.9487	4.6667	1.7692	1.5000	7.5500	4.3000	1.6500	1.7895	8.3684	5.0526	1.8947
22	24	1.6923	7.0513	4.0256	1.6410	1.5000	5.9500	3.4500	1.4500	1.8947	8.2105	4.6316	1.8421
23	25	1.8462	7.8462	4.9231	1.9744	1.6000	7.6500	4.9500	1.8000	2.1053	8.0526	4.8947	2.1579
24	26	2.0278	8.1111	4.5833	2.1111	1.7778	7.5556	4.0556	1.8889	2.2778	8.6667	5.1111	2.3333

Appendix Table C1 (Continued)

		Both sites				Pleasant Valley				Toledo			
Order	FN	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
25	27	2.2750	7.9250	5.0500	2.3500	2.1000	7.3000	4.8500	2.2000	2.4500	8.5500	5.2500	2.5000
26	28	1.9250	7.4250	4.3000	1.9750	1.8500	7.0000	4.2500	1.8000	2.0000	7.8500	4.3500	2.1500
27	29	1.8421	7.9211	5.1316	1.8947	1.6500	7.9500	5.2000	1.6500	2.0556	7.8889	5.0556	2.1667
28	30	1.8462	8.2564	4.9231	2.0000	1.9474	8.3684	5.2105	2.0526	1.7500	8.1500	4.6500	1.9500
29	31	1.7895	7.8684	4.1579	1.8684	1.4500	7.2000	3.8000	1.5500	2.1667	8.6111	4.5556	2.2222
30	32	2.0750	8.0750	4.9500	2.2250	1.7000	7.7500	4.7500	1.9500	2.4500	8.4000	5.1500	2.5000
31	33	2.1282	7.9744	4.7949	2.0769	2.0000	7.5000	4.6000	1.9000	2.2632	8.4737	5.0000	2.2632
32	34	1.8378	6.8649	4.1622	1.7568	1.7368	6.2105	3.7895	1.6842	1.9444	7.5556	4.5556	1.8333
33	35	1.7647	8.4706	5.0294	1.8235	1.5625	8.0625	5.3750	1.6875	1.9444	8.8333	4.7222	1.9444
34	36	1.8000	7.5250	4.4250	1.9000	1.6500	7.0000	4.2000	1.7500	1.9500	8.0500	4.6500	2.0500
35	37	1.7000	6.7250	3.8000	1.7500	1.6500	6.8500	3.7000	1.7500	1.7500	6.6000	3.9000	1.7500
36	38	1.7750	8.0000	4.7750	1.8750	1.7500	7.6000	4.1000	1.7500	1.8000	8.4000	5.4500	2.0000
37	39	1.9460	7.5135	4.3243	1.9460	2.0000	7.5000	4.5500	1.9500	1.8824	7.5294	4.0588	1.9412
38	40	1.8750	7.9250	4.5500	1.9500	2.1500	8.1000	4.6500	2.0000	1.6000	7.7500	4.4500	1.9000
39	41	1.6923	7.6154	3.9744	1.8718	1.5500	7.1000	3.7500	1.7500	1.8421	8.1579	4.2105	2.0000
40	42	1.9474	8.0263	4.6842	1.8684	1.9500	7.4500	4.7000	1.8500	1.9444	8.6667	4.6667	1.8889
41	43	1.8462	7.3333	4.6154	1.9231	1.5500	5.9000	3.7500	1.6000	2.1579	8.8421	5.5263	2.2632
42	44	2.0000	8.2069	5.1035	2.0690	1.6429	7.5000	5.0714	1.8571	2.3333	8.8667	5.1333	2.2667
43	45	1.7105	8.4737	5.0790	1.9737	1.6316	8.1579	5.2105	1.9474	1.7895	8.7895	4.9474	2.0000
44	46	1.9211	8.4737	4.8158	2.2632	2.1000	8.7000	4.5500	2.4000	1.7222	8.2222	5.1111	2.1111
45	47	2.4103	8.5385	5.2051	2.3590	2.4500	8.3500	5.1000	2.3500	2.3684	8.7368	5.3158	2.3684
46	48	1.9000	8.8000	5.3500	2.0250	1.8000	8.7000	5.2500	1.9500	2.0000	8.9000	5.4500	2.1000
47	49	1.7949	7.4103	4.5128	1.8462	1.7000	6.9500	4.1500	1.6500	1.8947	7.8947	4.8947	2.0526
48	50	2.1282	8.6154	5.0769	2.2308	2.0000	8.4000	4.5000	2.0000	2.2632	8.8421	5.6842	2.4737
49	51	1.8684	8.1579	4.9211	2.0000	1.8500	8.0500	4.7000	1.9500	1.8889	8.2778	5.1667	2.0556
50	52	1.8250	7.6500	4.3000	2.0000	1.8000	7.2000	3.8000	2.0000	1.8500	8.1000	4.8000	2.0000

Appendix Table C1 (Continued)

		Both sites				Pleasant Valley				Toledo			
Order	FN	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
51	53	1.8158	7.8684	4.5000	1.8421	1.8421	7.4737	4.3684	1.8421	1.7895	8.2632	4.6316	1.8421
52	54	1.8529	7.6177	4.3529	2.0000	1.7647	6.8235	4.0000	1.9412	1.9412	8.4118	4.7059	2.0588
53	55	1.7750	8.1250	4.4500	1.9250	1.7000	7.5500	4.0000	1.8500	1.8500	8.7000	4.9000	2.0000
54	56	2.1500	8.4750	5.1750	2.3500	1.7500	8.0000	4.6000	1.9500	2.5500	8.9500	5.7500	2.7500
55	57	1.8571	7.1786	3.5357	2.0357	1.6923	7.0000	3.4615	1.9231	2.0000	7.3333	3.6000	2.1333
56	58	2.0270	7.8919	4.9189	2.1351	1.8947	6.9474	4.3158	2.0000	2.1667	8.8889	5.5556	2.2778
57	59	1.8378	8.4054	5.1081	2.0000	1.6000	8.0000	5.0500	1.8000	2.1177	8.8824	5.1765	2.2353
58	**60**	2.0526	7.8421	4.8421	2.0263	2.0526	7.8947	4.8421	1.9474	2.0526	7.7895	4.8421	2.1053
59	**61**	2.0811	7.4054	4.3243	2.0000	1.9500	6.6500	3.9000	1.8500	2.2353	8.2941	4.8235	2.1765
60	**62**	2.2105	8.3684	5.0000	2.3158	2.0500	8.2500	5.0000	2.2000	2.3889	8.5000	5.0000	2.4444
61	**63**	1.7368	8.2632	4.9211	1.7895	1.7500	7.7500	4.3000	1.7500	1.7222	8.8333	5.6111	1.8333
62	**64**	1.7000	7.5500	4.6000	1.8250	1.4500	6.8000	4.0000	1.5500	1.9500	8.3000	5.2000	2.1000
63	**65**	1.5385	6.9487	4.1795	1.6154	1.5000	6.2000	3.9000	1.6000	1.5790	7.7368	4.4737	1.6316
64	**66**	1.7692	7.8462	4.7692	1.9744	1.4737	7.0526	4.1579	1.8421	2.0500	8.6000	5.3500	2.1000
65	**67**	1.9706	7.7941	4.6177	2.1471	1.7000	7.2000	4.6500	1.9500	2.3571	8.6429	4.5714	2.4286
66	**68**	1.8974	7.8205	4.4359	2.0256	1.8000	6.9500	3.9000	1.9000	2.0000	8.7368	5.0000	2.1579
67	**69**	1.7949	8.2821	5.2051	1.9744	1.6500	8.1500	5.2000	1.8500	1.9474	8.4211	5.2105	2.1053
68	**70**	1.9231	8.3846	5.1795	2.0000	1.8000	7.9500	4.8500	1.9000	2.0526	8.8421	5.5263	2.1053
69	**71**	1.6250	8.2250	4.8250	1.8250	1.4500	8.2500	4.9000	1.5500	1.8000	8.2000	4.7500	2.1000
70	**72**	2.2632	8.1579	5.0263	2.1842	2.2000	8.2000	5.0000	2.1000	2.3333	8.1111	5.0556	2.2778
71	**73**	1.7250	7.2250	4.4000	1.9000	1.6000	6.7000	3.9500	1.7000	1.8500	7.7500	4.8500	2.1000
72	**74**	1.8333	7.4167	4.3333	1.8889	1.6842	7.5263	4.5790	1.7368	2.0000	7.2941	4.0588	2.0588
73	**75**	2.0000	7.4500	4.4500	2.0500	1.8000	6.5000	3.8000	1.6500	2.2000	8.4000	5.1000	2.4500
74	**76**	1.8718	8.2051	4.9231	1.8974	1.9000	7.8500	4.9000	1.8500	1.8421	8.5790	4.9474	1.9474
75	**77**	1.8462	7.9231	4.7692	1.8205	1.5500	7.1000	4.4500	1.5000	2.1579	8.7895	5.1053	2.1579
76	**78**	2.0263	8.2632	4.8947	1.9737	1.7500	7.8000	4.5000	1.8000	2.3333	8.7778	5.3333	2.1667

Appendix Table C1 (Continued)

Order	FN	Both sites				Pleasant Valley				Toledo			
		NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
77	**79**	2.2500	8.1500	4.9750	2.1750	2.1500	7.7500	4.8500	2.0500	2.3500	8.5500	5.1000	2.3000
78	**80**	2.2000	8.0750	4.5750	2.2000	2.4000	7.5000	4.3500	2.3000	2.0000	8.6500	4.8000	2.1000
79	**81**	1.7180	7.8974	4.6410	1.9487	1.6000	7.7000	4.6000	1.8500	1.8421	8.1053	4.6842	2.0526
80	**82**	1.9706	8.3235	4.9118	1.9706	2.0000	8.3529	5.1765	1.8235	1.9412	8.2941	4.6471	2.1177
81	**83**	1.9250	8.6000	5.4250	2.1000	1.7500	8.4000	5.5000	1.9000	2.1000	8.8000	5.3500	2.3000
82	**84**	1.7692	7.6154	4.4872	1.7180	1.5500	7.4000	4.3000	1.4500	2.0000	7.8421	4.6842	2.0000
83	**85**	1.6316	7.1316	4.1579	1.8947	1.5000	6.3000	3.8000	1.8500	1.7778	8.0556	4.5556	1.9444
84	**86**	2.1000	8.8000	5.6750	2.1500	2.2500	8.7500	5.6500	2.1500	1.9500	8.8500	5.7000	2.1500
85	**87**	1.8462	7.7949	4.4103	1.9744	1.8500	7.5000	4.1000	1.9000	1.8421	8.1053	4.7368	2.0526
86	**88**	1.9744	8.4103	5.0256	2.1795	1.8500	8.2000	4.9000	2.2000	2.1053	8.6316	5.1579	2.1579
87	**89**	1.8611	8.0556	4.9167	1.9167	1.8500	7.9500	5.0000	1.9000	1.8750	8.1875	4.8125	1.9375
88	**90**	1.6000	6.7750	3.6750	1.8250	1.7000	6.8000	3.7500	1.8500	1.5000	6.7500	3.6000	1.8000
89	**91**	1.9231	8.4872	5.0513	2.0000	1.6500	8.1000	4.7000	1.7500	2.2105	8.8947	5.4211	2.2632
90	**92**	1.6410	6.7949	3.6410	1.8462	1.4500	5.4000	3.1000	1.6000	1.8421	8.2632	4.2105	2.1053
91	**93**	1.9487	7.9487	4.8974	2.0513	1.8000	7.0500	4.4000	1.9000	2.1053	8.8947	5.4211	2.2105
92	**94**	1.9189	8.3514	4.8649	1.9730	1.8000	7.8500	4.3000	1.8000	2.0588	8.9412	5.5294	2.1765
93	**95**	1.6750	7.8250	4.9250	1.9000	1.5000	7.1500	4.6000	1.7000	1.8500	8.5000	5.2500	2.1000
94	**96**	1.7632	7.5526	4.6842	1.7368	2.0000	7.2105	4.6316	1.7895	1.5263	7.8947	4.7368	1.6842
95	**97**	1.7436	7.3846	3.9744	1.7692	1.6500	6.5500	3.6000	1.6000	1.8421	8.2632	4.3684	1.9474
96	**98**	2.2051	8.4103	4.9744	2.2051	2.1500	8.3000	4.9000	2.2000	2.2632	8.5263	5.0526	2.2105
97	**99**	2.0000	8.4103	5.2821	2.1539	1.8421	8.1053	5.3158	2.0526	2.1500	8.7000	5.2500	2.2500
98	**100**	2.0000	8.4000	4.7250	2.1000	2.0500	8.0000	4.4000	2.1000	1.9500	8.8000	5.0500	2.1000
99	**101**	2.1250	8.6000	5.2250	2.2250	1.8000	8.2500	4.8000	2.0000	2.4500	8.9500	5.6500	2.4500
100	**102**	1.6410	7.2308	4.2308	1.8462	1.5263	7.3684	4.1053	1.6842	1.7500	7.1000	4.3500	2.0000
101	**103**	1.8421	7.7368	4.4737	1.8684	1.8500	7.4500	4.2000	1.8500	1.8333	8.0556	4.7778	1.8889
102	**104**	1.9167	7.3889	4.3611	2.1111	1.7368	6.2105	3.7368	1.8947	2.1177	8.7059	5.0588	2.3529

Appendix Table C1 (Continued)

		Both sites				Pleasant Valley				Toledo			
Order	FN	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
103	**105**	1.7000	5.2000	3.3000	1.6500	1.2000	3.3000	2.3000	1.1500	2.2000	7.1000	4.3000	2.1500
104	**106**	1.6667	7.6944	4.5278	1.7778	1.5263	7.8421	4.6842	1.5790	1.8235	7.5294	4.3529	2.0000
105	**107**	1.8421	7.7368	4.5526	2.0000	1.6500	6.8500	4.1500	1.8500	2.0556	8.7222	5.0000	2.1667
106	**108**	2.0769	8.2051	4.7180	2.1539	1.8000	7.8500	4.3500	1.8500	2.3684	8.5790	5.1053	2.4737
107	**109**	2.2571	7.8857	4.4857	2.2286	2.1765	7.6471	4.8235	2.2941	2.3333	8.1111	4.1667	2.1667
108	**110**	2.0750	8.1250	4.4000	2.1500	1.9500	7.5500	4.1000	2.0500	2.2000	8.7000	4.7000	2.2500
109	**111**	2.0278	8.0556	4.7500	2.1389	1.8947	7.2105	4.2632	1.8947	2.1765	9.0000	5.2941	2.4118
110	**112**	1.7632	8.5526	5.1316	1.9474	1.7368	8.3684	5.1053	1.8421	1.7895	8.7368	5.1579	2.0526
111	**113**	1.5526	6.6842	3.4737	1.5263	1.3000	6.0500	2.9000	1.4000	1.8333	7.3889	4.1111	1.6667
112	**114**	1.7436	8.3077	4.7949	2.0256	1.8000	7.9500	4.3500	2.1000	1.6842	8.6842	5.2632	1.9474
113	115	1.5750	7.8500	4.1000	1.7500	1.4500	6.9500	4.0000	1.6500	1.7000	8.7500	4.2000	1.8500
114	116	1.7778	7.6944	4.2500	1.8056	1.7368	8.0000	4.5263	1.7895	1.8235	7.3529	3.9412	1.8235
115	117	1.7105	8.1053	5.0000	1.7368	1.6500	8.2500	5.2500	1.6000	1.7778	7.9444	4.7222	1.8889
116	118	2.0000	8.6667	5.2051	2.2051	1.6500	8.5500	4.9000	1.9000	2.3684	8.7895	5.5263	2.5263
117	119	1.8684	7.7368	4.7895	1.9211	1.9500	7.5000	4.4500	1.9500	1.7778	8.0000	5.1667	1.8889
118	120	1.7353	7.1765	3.7059	1.7941	1.6111	6.1667	2.9444	1.7222	1.8750	8.3125	4.5625	1.8750
119	121	1.8205	8.3077	5.0000	1.8718	1.4737	8.0000	4.6316	1.5790	2.1500	8.6000	5.3500	2.1500
120	122	1.8158	7.7632	4.6316	1.9211	1.6000	7.5000	4.3000	1.8500	2.0556	8.0556	5.0000	2.0000
121	123	1.9487	8.2564	5.0513	2.1539	1.9000	7.9500	4.9000	1.9500	2.0000	8.5790	5.2105	2.3684
122	124	1.6923	6.7949	4.0769	1.7436	1.5500	5.4000	3.5000	1.4500	1.8421	8.2632	4.6842	2.0526
123	125	1.6487	7.6757	4.3784	1.7568	1.8000	7.9500	4.7500	1.9000	1.4706	7.3529	3.9412	1.5882
124	126	2.1111	7.8611	4.8056	2.1389	2.1053	7.1053	4.2632	2.1053	2.1177	8.7059	5.4118	2.1765
125	127	1.8462	8.3846	5.1282	1.9231	1.7500	8.1500	4.9500	1.8500	1.9474	8.6316	5.3158	2.0000
126	128	1.7297	7.8108	4.6487	1.8378	1.5500	7.4500	4.7500	1.7000	1.9412	8.2353	4.5294	2.0000
127	129	1.7000	7.9000	4.6250	1.8000	1.6500	7.8500	4.8500	1.7500	1.7500	7.9500	4.4000	1.8500
128	130	2.0250	8.4250	4.9750	2.0250	1.8000	8.3000	4.8500	1.9000	2.2500	8.5500	5.1000	2.1500

Appendix Table C1 (Continued)

		Both sites				Pleasant Valley				Toledo			
Order	FN	NC	NR	FD	FC	NC	NR	FD	FC	NC	NR	FD	FC
129	131	1.8000	8.2750	4.6250	2.0250	1.6500	8.0500	4.3500	1.9500	1.9500	8.5000	4.9000	2.1000
130	132	1.9730	8.1351	5.0000	2.0270	1.8000	8.0500	5.1000	1.8500	2.1765	8.2353	4.8824	2.2353
131	133	1.8158	7.7895	4.7105	1.8421	1.6000	6.9500	4.4500	1.5500	2.0556	8.7222	5.0000	2.1667
132	134	1.7895	7.6316	4.5790	1.9211	1.7500	7.4500	4.5000	1.9000	1.8333	7.8333	4.6667	1.9444
133	135	1.5000	4.7083	2.8750	1.5000	1.4546	3.5455	2.2727	1.4546	1.5385	5.6923	3.3846	1.5385
134	136	1.7949	7.9487	5.0000	1.9487	1.6500	7.4000	4.6500	1.8500	1.9474	8.5263	5.3684	2.0526
135	137	1.9737	8.2368	5.1053	2.1053	1.8947	7.7368	4.8947	2.0000	2.0526	8.7368	5.3158	2.2105
136	138	1.7143	8.3143	4.5714	1.7714	1.8421	8.0000	4.5263	1.7895	1.5625	8.6875	4.6250	1.7500
137	139	1.6389	8.3056	4.8889	1.7500	1.4737	8.2105	4.9474	1.6316	1.8235	8.4118	4.8235	1.8824
138	140	1.5526	7.4211	4.4737	1.6316	1.5500	6.8500	4.3000	1.5500	1.5556	8.0556	4.6667	1.7222
139	141	1.6410	8.3846	5.1539	1.8205	1.4500	8.1500	5.2000	1.7500	1.8421	8.6316	5.1053	1.8947
140	142	1.6216	7.2703	3.9460	1.6757	1.3889	6.3333	3.3333	1.5556	1.8421	8.1579	4.5263	1.7895
141	143	1.6410	7.1539	4.6667	1.7692	1.3684	6.5263	4.5790	1.5263	1.9000	7.7500	4.7500	2.0000
142	144	1.7632	7.8684	4.7632	1.9211	1.7500	7.4500	4.5000	1.9000	1.7778	8.3333	5.0556	1.9444
143	145	1.6250	6.8250	4.1750	1.8000	1.4000	6.1000	3.6500	1.5000	1.8500	7.5500	4.7000	2.1000
144	146	2.0294	8.3529	4.9118	2.0588	1.8889	7.8889	4.6111	1.9444	2.1875	8.8750	5.2500	2.1875
145	147	1.8750	7.6500	4.6750	1.9000	1.6000	6.5000	3.9500	1.7000	2.1500	8.8000	5.4000	2.1000
146	148	1.9000	8.2500	4.7500	1.8250	1.8000	7.5500	4.3000	1.6500	2.0000	8.9500	5.2000	2.0000
147	149	2.0571	7.7143	4.5429	2.1429	2.1500	7.8000	4.6500	2.3500	1.9333	7.6000	4.4000	1.8667
148	150	1.9487	8.2564	4.7692	1.8974	1.8500	7.6500	4.2000	1.6500	2.0526	8.8947	5.3684	2.1579
149	151	1.8718	8.1539	5.0769	1.9487	1.7000	7.9500	4.8500	1.8000	2.0526	8.3684	5.3158	2.1053
150	152	1.7692	7.1282	4.1026	1.8718	1.7000	6.2000	3.6500	1.7500	1.8421	8.1053	4.5790	2.0000
151	153	1.8611	8.1389	4.6944	1.8611	1.8000	7.8000	4.4500	1.7500	1.9375	8.5625	5.0000	2.0000
152	154	2.0270	7.8378	4.4595	2.1081	1.7500	7.0500	3.8000	1.8500	2.3529	8.7647	5.2353	2.4118

Appendix Table C2. ANOVA tables of SNC traits at Pleasant Valley.

Needle Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	7.869685	2.623229	4.65	0.0032
Family	151	139.928323	0.926678	1.64	<0.0001
Rep*Family	453	255.357471	0.563703	1.22	0.0027
Error	2356	1091.483333	0.463278		
Total	2963	1494.638812			

Foliage Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	10.212371	3.404124	7.60	<0.0001
Family	151	125.126851	0.828655	1.85	<0.0001
Rep*Family	453	202.875937	0.447850	1.38	<0.0001
Error	2356	765.583333	0.324950		
Total	2963	1103.798491			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	808.822435	269.607478	40.43	<0.0001
Family	151	1929.124066	12.775656	1.92	<0.0001
Rep*Family	453	3021.013700	6.668904	1.79	<0.0001
Error	2356	8761.633330	3.718860		
Total	2963	14520.593530			

Foliage Density

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	269.261761	89.753920	28.81	<0.0001
Family	151	950.469908	6.294503	2.02	<0.0001
Rep*Family	453	1411.388865	3.115649	1.74	<0.0001
Error	2356	4214.050000	1.788646		
Total	2963	6845.170534			

Appendix Table C3. ANOVA tables of SNC traits at Toledo.

Needle Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	27.701702	9.233901	15.60	<0.0001
Family	151	130.279713	0.862780	1.46	0.0016
Rep*Family	453	268.097191	0.591826	1.40	<0.0001
Error	2206	935.200000	0.423935		
Total	2813	1361.278606			

Foliage Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	14.187701	4.729234	9.21	<0.0001
Family	151	115.478379	0.764757	1.49	0.0009
Rep*Family	453	232.672497	0.513626	1.53	<0.0001
Error	2206	741.283333	0.336031		
Total	2813	1103.621911			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	93.839057	31.279686	9.06	<0.0001
Family	151	751.181202	4.974710	1.44	0.0022
Rep*Family	453	1564.260873	3.453115	1.38	<0.0001
Error	2206	5525.116667	2.504586		
Total	2813	7934.397799			

Foliage Density

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Replication	3	44.892441	14.964147	5.19	0.0016
Family	151	559.517334	3.705413	1.28	0.0259
Rep*Family	453	1306.310711	2.883688	1.65	<0.0001
Error	2206	3851.600000	1.745966		
Total	2813	5762.320486			

Appendix Table C4. ANOVA tables of SNC traits over the two sites.

Needle Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	79.571263	79.571263	13.27	<0.05
Replication(Site)	6	35.571387	5.928565	10.26	<0.0001
Family	151	173.182509	1.146904	1.78	0.0002
Site*Family	151	97.442212	0.645313	1.12	0.1761
Rep*Family(Site)	906	523.454662	0.577765	1.30	<0.0001
Error	4562	2026.683333	0.444253		
Total	5777	2935.905366			

Foliage Color

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	98.295007	98.295007	23.79	<0.01
Replication(Site)	6	24.400072	4.066679	8.46	<0.0001
Family	151	157.632661	1.043925	1.92	<0.0001
Site*Family	151	82.308974	0.545093	1.13	0.1458
Rep*Family(Site)	906	435.548434	0.480738	1.46	<0.0001
Error	4562	1506.866667	0.330308		
Total	5777	2305.051815			

Needle Retention

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	1159.655068	1159.655068	7.68	>0.05
Replication(Site)	6	902.661491	150.443382	29.73	<0.0001
Family	151	1805.810283	11.959009	2.14	<0.0001
Site*Family	151	844.316817	5.591502	1.10	0.2003
Rep*Family(Site)	906	4585.274573	5.061009	1.62	<0.0001
Error	4562	14286.750000	3.131686		
Total	5777	23584.468230			

Foliage Density

Source	*DF*	*Type III SS*	*MS*	*F-value*	*p-value*
Site	1	349.650942	349.650942	6.66	>0.05
Replication(Site)	6	314.154202	52.359034	17.45	<0.0001
Family	151	1022.819487	6.773639	2.17	<0.0001
Site*Family	151	471.542573	3.122798	1.04	0.3615
Rep*Family(Site)	906	2717.699575	2.999668	1.70	<0.0001
Error	4562	8065.650000	1.768007		
Total	5777	12941.516780			