differences are expressed, they could be transmitted to the next generation as well.


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### Literature


Bjørnstad, Å.: Photoperiodical after-effects of parent plant environment in Norway spruce (*Picea abies* (L.) Karst.) seedlings. Rep. Norw. For. Res. Inst. **36**, 1—30 (1981). —— Dormling, I., Gustavsson, Å. and von Wettstein, D.: The experimental control of life cycle in *Picea abies* (L.) Karst. I. Some basic experiments on the vegetative cycle. Silvae Genet. **17**, 44—64 (1968). —— Dormling, I. and Johnsen, Ø.: Effects of the parental environment on full-sib families of *Pinus sylvestris*. Can. J. For. Res. **22**, 88—100 (1992). —— Ellis, R. H.: Seed and seedling vigor in relation to crop growth and yield. Plant Growth Reg. **11**, 249—255 (1992). —— Heide, O. M.: Growth and dormancy in Norway Spruce ecotypes (*Picea abies*). I. Interaction of photoperiod and temperature. Physiol. Plant. **30**, 1—12 (1974). —— ISTA: International Rules for Seed Testing. Rules 1993, International Seed Testing Association. Seed Sci. and Technol., Supplement 21, 1—288 (1993). —— Johnsen, Ø.: After-effects on progenies from orchard clones moved to non-native environments. *In:* Physiology and genetics of reforestation. Eds. Worral, J., Loo-Dinkins, J. and Lester, D. P. Proc. of The 10th North American Forest Biology Workshop, Vancouver, B. C.: pp 1—11 (1988). —— Johnsen, Ø.: Phenotypic changes in progenies of northern clones of *Picea abies* (L.) Karst. grown in a southern seed orchard. I. Frost hardiness in a phytotron experiment. Scand. J. For. Res. **4**, 317—330 (1989a). —— Johnsen, Ø.: Phenotypic changes in progenies of northern clones of *Picea abies* (L.) Karst. grown in a southern seed orchard. II. Seasonal growth rythm and height in field trials. Scand. J. For. Res. **4**, 331—341 (1989b). —— Johnsen, Ø., Dietrichson, J. and Skaret, G.: Phenotypic changes in progenies of northern clones of *Picea abies* (L.) Karst. grown in a southern seed orchard. III. Climatic damage and growth in a progeny trial. Scand. J. For. Res. **4**, 343—350 (1989). —— Johnsen, Ø., Haug, G., Apeland, I. and Østreng, G.: Sexual reproduction in a greenhouse reduces autumn frost hardiness of *Picea abies* progenies. Tree Physiol., in press (1994). —— Johnsen Ø. and Østreng, G.: Effects of plus tree selection and seed orchard environment on progenies of *Picea abies*. Can. J. For. Res. **24**, 32—38 (1994). —— Koski, V.: Adaption of trees to the variation in the length of the growing season. In: Kaurin, Junttila and Nielsen (Eds.). Plant production in the North., 267—275 (1985). —— Koski, V. and Sievänen, R.: Timing of growth cessation in relation to the variation in the growing season. In: Tigerstedt, Puttonen and Koski (Eds.). Crop physiology of Forest Trees, 167—191 (1985). —— Lindgren, D. and Wei, R. P.: Effects on maternal environment on mortality and growth in young *Pinus sylvestris* field trials. Tree Physiol. **14**, 323—327 (1994). —— Magnesen, S.: Ecological experiments regarding growth termination in seedlings of Norway spruce. I. Effects of daylength and temperature conditions during growth season. (Norwegian with English summary). Meddr.. Vestl. for. ForsøksSta. **14**(1), 1—50 (1969). —— Perry, T. O.: Dormancy of trees in winter. Science. **171**, 29—36, (1971). —— Perry, T. O.: Maternal effects on early performance of tree progenies. *In:* Tree physiology and tree improvement. *Eds.* Cannell, M. G.-R and Last, F. T. Academic Press, London. pp 473—481 (1976). —— Rehfeldt, G. E.: Growth and cold hardiness of intervariental hybrids of Douglas-fir. Theor. Appl. Genet. **50**, 3—15 (1977). —— Robak, H.: On the connection between day length and termination of the annual growth period in seedlings of some conifers of interest to Norwegian forestry. (Norwegian with Englisch summary). Meddr. Vestl. for. ForsøksSta. **10**, 1—62 (1957). —— SAS Institute Inc.: SAS/STAT Users's guide. Version 6. 4th edition. Vol 2 SAS Inst.. Inc., Cary, NC: 846 p (1989). —— Skrøppa, T.: The seed weight did not affect first year's bud-set in *Picea abies*. Scand. J. For. Res. **3**, 437—439 (1988) —— Skrøppa, T.: Growth rhythm and hardiness of *Picea abies* progenies of high altitude parents from seed produced at low elevations. Silvae Genet. **43**, 95—100 (1994). —— Skrøppa, T. and Johnsen, Ø.: The genetic response of plant populations to a changing environment: the case for non-Mendelian processes. NATO ASI Series, Vol. I. 20 Biodiversity, Temperate Ecosystenme and Global Change. (Eds.. Boyle, T. J. B. and Boyle, C.E.B.). Springer-Verlag, Berlin, Heidelberg. 183—199 (1994).


# Screening Pinus sylvestris for Resistance to Sphaeropsis sapinea


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### Summary

Artificial inoculation was used to determine if it is feasible to screen for resistance to *Sphaeropsis sapinea* (Fr.) Dyko and Sutton in 2-year old seedlings of *Pinus sylvestris* L. Six varieties were exposed to 2 spore concentrations in outdoor mist chambers. Inoculations were repeated on 4 dates in late spring to early summer.

Varieties differed in infection incidence, indicating potential resistance. Phenological growth stage of seedlings was more important than date of inoculation; infection levels decreased after needles began to elongate. Therefore inoculations would be most effective during active shoot elongation prior to needle elongation. At least 2 inoculations are recommended when screening for resistance with this method because the incidence of infection varied among growth stages, and varieties differed in growth periodicity.

The Penn State XP-74 variety was found to be more resistant than the others, and the East Anglia variety was the most susceptible after varietal infection rates were adjusted for variation due to stage of growth and health of seedlings. The evidence for resistance suggests that genetic improvements may be attained through this screening method.



*Key words: Pinus sylvestris, Sphaeropsis sapinea, Diploida pinea,* disease resistance, selection method.

*FDC*: 165.53; 443.2; 165.62; 172.8 *Sphaeropsis sapinea;* 174.7 *Pinus sylvestris.*


### Introduction

*Sphaeropsis sapinea* (Fr.) Dyko and Sutton (=*Diploida pinea* [Desm.] Kickx.) causes a variety of diseases including seedling collar rot, tip and twig blight and, on weakened or injured trees, stem cankers and stem and branch dieback. Conifers in eight genera are susceptible to this fungus, but it is most common in *Pinus*. Pycnidia can be found singly or in clusters on bark, cone scales, and leaves. Conidia initially are yellowish-brown, darken with maturity, and occasionally are uniseptate (Haddow


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*Sphaeropsis sapinea* is common in conifer plantations throughout the world, especially on non-native species and trees planted in stressful locations. The pathogen is a serious threat to the Christmas tree industry in North America. One commonly grown tree, Scotch pine *(Pinus sylvestris L.)*, is highly susceptible. Several studies have demonstrated that natural resistance to *S. sapinea* occurs within conifer species (SWART et al., 1988; BURDON et al., 1982; WRIGHT et al., 1976).

The primary objective of this study was to determine the feasibility of using artificial inoculation to screen Scotch pine seedlings for resistance to *S. sapinea* using varieties representative of those in the Christmas tree industry. In addition, we wanted to determine the optimal growth stage for inoculation, develop a method for inoculum production, and explore if the concentration of spores influences inoculation results.

## Materials and Methods

*Sphaeropsis sapinea Collection and Culture*

Scotch pine shoots killed by *S. sapinea* were collected for inoculum culture in January from 2 locations in central Pennsylvania. Twig samples were collected from single trees of 5 varieties to allow for more variation in the inoculum, because WANG et al. (1985) found variation in pathenogenicity among sources.

In the laboratory, pycnidia picked out of the needles with a probe were crushed, and spores were streaked onto a water agar plate. After the spores germinated (2 to 4 hours), individual spores were transferred to 2% malt ager plates, which then were placed under fluorescent lights set at a 12-hour photo period. The cultures grew well with no contamination, but produced no pycnidia even after being overlaid with Austrian pine *(P. nigra* ARNOLD) needles (PALMER, et al., 1987) to aid in production of fruiting bodies in culture, or after transfer to a low nutrient medium consisting of five milliliters of potato dextrose agar per liter of water agar overlaid with Austrian pine needles. So the light period was increased to 24 hours per day and the plates were sealed with parafilm to increase humidity. Fruiting bodies developed 12 days after these changes were made. Additional transfers were made to low nutrient agar to provide ample cultures for inoculation and to maintain the different isolates.

*Pathogenic Strains*

To examine the possibility that different strain types were present, needles containing pycnidia were taken from one-month-old cultures of each inoculum source and fixed in gluteraldehyde and osmium tetroxide. Samples were dehydrated using a gradient ethanol series and left overnight in 100% ethanol, then frozen and fractured using liquid nitrogen and critical-point dried in a Polaron E3000. Needle fragments were mounted on aluminium stubs, sputter-coated with gold, and the spore surfaces were examined with an ISI 60 Scanning Electron Microscope to determine whether they were smooth or pitted.

*Experimental Conditions*

Controlled variables in this study were 4 inoculation dates, 6 varieties of *P. sylvestris*, and 2 *S. sapinea* spore concentrations. Health level and growth stage of each tree at the time of inoculation were recorded to help understand variation in infection levels. The inoculation dates were 1, 6, 12, and 22 June 1990. The initial inoculation occurred when at least 25 % of the seedlings of each variety had broken bud. The next 3 inoculations were at approximately weekly intervals, varying according to the weather. The 6 varieties used were Commercial French, East Anglia, Spanish Guadarrama, Pennspanish, Penn State XP-74, and Penn State XP-75; all of these are being grown in commercial nurseries. The last 3 are improved varieties containing various provenance hybrids, and are produced in seed orchards. Two-year-old seedlings obtained from Carino Nurseries, Indiana, PA, were planted in 1 gallon pots 45 days before the first inoculation.

Seedlings to be inoculated were placed temporarily in 2 open-topped chambers located outside. The chambers were 2.5 meters by 3.1 meters by 1.2 meters high with plastic wrapped around the sides of a wooden frame. A misting system suspended about 0.6 meters above the potted seedlings misted for 10 seconds at 3-minute intervals.

Seedlings were classified into 5 pre-inoculation health levels and 15 growth stages, to examine whether any level of health or stage of growth might be more susceptible than others. The health levels were based mainly on the percentage of needles that were chlorotic or brown-tipped, ranging from none to more than 75 % in 25 % increments. The growth stages took into account the elongation of the terminal shoot in percent of new growth and the elongation of needles in cm. Any trees that appeared to be dead or dying at the start of the inoculation period were excluded.

Fifteen trees of each variety were placed in each of the 2 chambers on each of the 4 inoculation dates, arranged in 15 blocks of 6 trees containing 1 tree of each variety. Altogether, 90 trees were inoculated in each chamber on each date, except the fourth date when only 20 XP-75 trees were available for inoculation. A third chamber, placed between the 2 employed for inoculation, served as the control where 5 trees of each source were arranged in the same manner but not inoculated. The same control trees were used each time, but removed from the chamber between inoculation dates. The purpose of the controls was to determine if inoculum was being introduced from any source other than artificial inoculation.

*Inoculation Method*

Two spore suspensions were prepared on each inoculation date from 30 needles in culture, 6 from each inoculum source. If the sources differ in virulence, the mixed inoculum would tend to increase the likelihood of infection, and sacrifice some specificity of information about reactions. A #300 wire mesh screen was used to filter out most of the unwanted fungal material. The concentration of spores in suspension was determined using a hemocytometer, and adjusted as necessary.

Seedlings in one chamber were inoculated with a concentration of 5,000 spores per ml, and with 2,500 spores per ml in the other. The spore suspension was transported in an ice bath to prevent premature germination. Inoculum was sprayed on the seedlings with a DEVILBISS atomizer. Each bud cluster or emerging growth was sprayed with 3 squirts equivalent to about 0.1 ml each. Seedlings remained in the misting chambers for 48 hours after inoculation, the maximum wetness duration recommended by CHOU (1982a), and were then moved to a nearby field for observation.

*Table 1.* — Analysis of variance in infection after artificial inoculation of 6 varieties of Scotch pine with 2 inoculum concentrations on 4 dates.

| Source of variation | df | F value | Pr>F |
|---|---|---|---|
| Date | 3 | 1.48 | 0.206 |
| Variety | 5 | 4.64 | 0.000 |
| Concentration | 1 | 5.80 | 0.016 |
| Date x Variety | 15 | 2.09 | 0.004 |
| Date x Concentration | 3 | 0.97 | 0.409 |
| Variety x Concentration | 5 | 2.47 | 0.031 |

*Data Collection and Analysis*

Stage of growth and health level were recorded on the seedlings of each inoculation group at 2-week intervals, both before and after inoculation. Response to inoculation was determined by examining the top whorl of shoots. A seedling was considered infected if it remained alive and one or more dead shoots exhibited a distinct crook, in many cases the typical „shepherd's crook" (SINCLAIR et al., 1987). A seedling was considered uninfected if all shoots remained alive. Seedling responses to inoculation were examined periodically, with a final examination at the end of July when the seedlings were classified as infected or uninfected. Random samples of dead shoots from 40 seedlings classified as infected were taken to determine if *S. sapinea* was present. The analysis of variance in the incidence of infection was calculated based on a completely randomized design using numbers of seedlings infected in proportion to the size of each group, without transformation of data. Inoculation date, spore concentration, and variety were the sources of variation.

As the data were examined, it was found via $\chi^2$ tests that different varieties exhibited unequal mortality and uneven distribution of seedlings among health classes and stages of growth. To correctly attribute resistance, it was necessary to account for these differences among varieties. Incidence of infection was calculated by using data only from seedlings in those growth stages and health categories that were most likely to be infected over all the varieties.

## Results

Inoculum was successfully produced through collection and culture of *Sphaeropsis sapinea* fruiting bodies. Identification of the spore type as suggested by WANG et al. (1985) was ambiguous. Although the samples were taken from cultures started from single spores, some pycnidia contained both pitted and smooth spores. Spores without surface pitting appeared to be more common. Both types of spores were present in the suspensions used for artificial inoculation.

*S. sapinea* was found in 100 % of samples taken from symptomatic, inoculated trees. None of the control trees showed symptoms of being infected by *S. sapinea,* and no signs of infection were found in samples taken from the control trees.

More seedlings were infected at the higher spore concentration (*Tables 1* and *2*). Over all varieties and dates, 16 % of seedlings inoculated with 2,500 spores/milliliter were infected, whereas 21 % of seedlings inoculated with 5,000 spores/milliliter were infected. Of the individual varieties, Penn State's XP-74 had the lowest average infection rate at 8 % and East Anglia had the highest with 30 % infected. Pennspanish, XP-74, and XP-75 had slightly greater infection levels at the higher spore concentration. Commercial French and East Anglia had many more infected seedlings at the higher concentration, whereas Spanish Guadarrama had a higher percentage at the lower concentration (*Table 2*).

Date of inoculation did not significantly affect the percentage of seedlings infected (*Table 1*), but stage of growth at time of inoculation did (*Table 3*, $\chi^2 = 210.0$, 9df, $P< 0.0001$). Seedlings inoculated during active shoot elongation, i.e. from stage 2 to stage 7, had a greater percentage of infection (*Table 3*). Eighteen percent of inoculated seedlings were in these growth stages, which had an average infection level of 45 %. Over 45 % of seedlings were inoculated during needle elongation (stage of growth 10 to 14), but this group had an average of only 9% infection. Seedlings inoculated prior to active shoot elongation and

*Table 3.* — Number of Scotch pine seedlings in each stage of growth at time of inoculation and percentage of seedlings infected.

| Stage of Growth[1] | Total Number | Percent Infected |
|---|---|---|
| 0 & 1 | 136 | 19 |
| 2 | 18 | 44 |
| 3 | 10 | 90 |
| 4 | 13 | 31 |
| 5 | 22 | 45 |
| 6 | 25 | 32 |
| 7 | 28 | 46 |
| 8 | 29 | 17 |
| 9 | 56 | 21 |
| 10-14 | 297 | 9 |
| All Stages | 634 | 19 |

[1]) Growth stages defined as 0 — no bud elongation, 2 — bud elongation started, 2 to 4 — shoots elongated up to 25%, 50%, or 75%, 5 to 7 — rapid growth finished and bud scales peeled from 25%, 50%, or 75% of new growth, 8 to 9 — needles emerged from fascicle sheaths on lower or upper half of new growth, 10 to 14 — needles on new shoots elongated 0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm, or 2.5+ cm.

*Table 2.* — Percentage of seedlings infected in 6 varieties of Scotch pine inoculated with 2 dosages of *Sphaeropsis sapinea* spores.

| | Inoculum Concentration | | |
|---|---|---|---|
| Variety | 5,000 sp/ml | 2,500 sp/ml | Overall |
| Penn State XP-74 | 8a[1] | 7a | 8a |
| Spanish Guadarrama | 9a | 15a | 11ab |
| Commercial French | 25b | 12a | 19abc |
| Pennspanish | 22ab | 20a | 21bc |
| Penn State XP-75 | 28b | 22a | 26c |
| East Anglia | 36b | 23a | 30c |
| All Varieties | 21 | 16 | 19 |

[1]) Items with the same letter do not differ significantly, P = 0.05.

*Table 4.* — Total number of Scotch pine seedlings in each health class at time of inoculation and percentages of survival and infection.

| Health Class[1] | Total Number | Percent Survival | Percent Infected |
|---|---|---|---|
| 1 | 94 | 100 | 16 |
| 2 | 307 | 93 | 17 |
| 3 | 164 | 82 | 21 |
| 4 | 57 | 50 | 25 |
| 5 | 12 | 8 | 50 |

[1]) Health classes defined as 1 — excellent condition with no chlorosis or brown needle tips, 2 — good condition with less than 25% of needles discolored, 3 — fairly good health with 25% to 50% of needles discolored, 5 — poor condition with 75% to 100% of needles chlorotic or brown tipped, but buds alive.

during early needle emergence (stages of growth 1, 8 and 9), had infection rates ranging from 17 % to 21 %.

Less healthy trees were more likely to become infected (*Table 4*), as health at time of inoculation had a significant effect on percentage of infection ($\chi^2 = 40.9$, 4df, $P<0.0001$). Fifty % of seedlings in health class 5 were infected, while seedlings in health classes 1 through 4 ranged from 16 % to 25 % infection.

Due to the influence of both stage of growth and health on the incidence of infection, revised comparisons among varieties were based upon percentage of infected seedlings that were in stages of growth 2 to 7 with a health rating of 1 to 3 (*Table 5*).

Two first-level interactions also were significant, spore concentration by variety and inoculation date by variety (*Table 1*). The reason for the spore concentration by variety interactions is unknown, but the inoculation date by variety interaction can possibly be explained by differing varietal growth patterns.

## Discussion

Some plantations have been so severely damaged by *S. sapinea* in New Zealand and Africa that total tree production was lost (Swart and Wingfield, 1991; Zwolinski et al., 1990; Chou, 1976a). In view of such high natural infection levels, artificial inoculation techniques used to screen for resistance should seek to produce the highest infection incidence possible in susceptible trees. Some studies have achieved levels approaching 100 % infection (Swart et al., 1988; Chou, 1982a; Burdon et al., 1982; Chou, 1976a).

Swart et al. (1988) obtained 40 % to 50 % infection on non-wounded, artificially inoculated *P. pinaster* seedlings and 80 % to 100 % on wounded seedlings. This demonstrated that high infection levels can be produced artificially, especially with wounding. Higher inoculum concentrations result in higher percentages of infection (Chou, 1976b), as was found in this experiment. Most published studies have used 10,000 spores/ml to 15,000 spores/ml, but have used different methods of application than in this study and have not reported the amount applied per tree (Swart et al., 1988; Burdon et al., 1982; Chou, 1982b). Concentrations up to 5,000 spores/ml were used in this study, with spore loads of up to 500 spores sprayed on each seedling.

The inoculation method used for resistance screening should be quantifiable and reproducible. Calculation of spore loads would allow for comparison between artificial inoculation and field conditions. Unfortunately, no one has found an accurate method for determining natural *S. sapinea* spore levels. Brookhouser and Peterson (1971) used petrolatum-coated microscope slides to capture spores, but this method has been regarded as inaccurate by others (Gregory, 1973; Ingold, 1971).

Infection levels obtained in this experiment ranged from 7 % to 36 % among varieties exposed to 2 spore concentrations (*Table 2*). All but one variety had greater infection levels at the higher spore concentration. The reason that infection of Spanish Guadarrama was higher at the lower spore concentration is unknown. Inefficient spore application and exposure of most trees at less susceptible stages are the mot likely explanations of the relatively low infection levels. Results of this study and others suggest that higher spore loads likely would produce even greater incidence of infection.

The stage of seedling growth at inoculation was critical in seedling susceptibility to infection. Infection was highest during shoot elongation prior to needle emergence (stages of growth 2 to 7), with an average of 45 % infected seedlings (*Table 3*). These growth stages should be the focus of future inoculations. Trees just starting to grow (growth stage 1) and those whose needles were actively expanding (growth stages 8 to 14) had levels of infection that were much lower, so these stages should be excluded from future screening inoculations.

The 6 varieties had different growth patterns. On average, East Anglia seedlings flushed first and finished growth before any of the other varieties. East Anglia seedlings also had the largest average height increase of any of the varieties. Commercial French seedlings also flushed early, but finished growth about the same time as the other 4 varieties. Spanish Guadarrama seedlings flushed earlier than the 3 Penn State varieties, had the smallest average height increase and were generally slower growing than the other 5 varieties. The 3 Penn State varieties (Pennspanish, XP-74, and XP-75) had very similar

*Table 5.* — Total infection percentages of Scotch pine varieties compared to infection percentages in health groups 1 to 3 and growth stages 2 to 7.

| Variety | Total Percent Infected | Percent Infected In: Health Groups 1-3 | Growth Stages 2-7 | Health Groups 1-3 & Growth Stages 2-7 |
|---|---|---|---|---|
| Penn State XP-74 | 8a[1] | 7 | 21 | 21a |
| Spanish Guadarrama | 11ab | 11 | 30 | 32ab |
| Commercial French | 19abc | 18 | 47 | 36ab |
| Pennspanish | 21bc | 21 | 41 | 42ab |
| Penn State XP-75 | 26c | 25 | 67 | 63b |
| East Anglia | 30c | 27 | 67 | 67b |

[1]) Percentages followed by the same letter do not differ significantly, P = 0.05.

growth patterns after an initial delay in flushing by XP-75.

Consistent with the differing growth patterns among varieties, the highest infection levels of the varieties were reached on different dates. On each of the 4 inoculation dates, it was possible for one variety to have more seedlings in the most susceptible stages of growth than another variety, many of whose seedlings had either not reached or had surpassed that stage of growth. This can explain much of the infection variation among dates caused by stage of growth, resulting in the date by variety interaction.

Health of seedlings prior to inoculation was important, because trees that were less healthy had significantly higher infection levels. Although infection rate increased as health decreased (*Table 4*), the only significant difference was between health class 5 and all others.

When the infection levels are considered over the most susceptible stages of growth and the most resistant health classes, more useful resistance comparisons are obtained (*Table 5*). After adjustment for health and growth stages, there were still significant differences in susceptibility among the varieties, even though the number of seedlings providing data was reduced greatly. Penn State's XP-74 experimental variety had a significantly lower percentage of infected trees (21 %) than either Penn State's XP-75 (63 %) or East Anglia (67 %) (*Table 5*). Spanish Guadarrama. Commercial French, and Pennspanish were not statistically different from the other three varieties. The rank of varietal susceptibility did not change as a result of the adjustment.

Because infection incidence did not significantly differ over inoculation dates, spanning 23 days during emergence and elongation of new growth, inoculations could take place on one or several dates. However, stage of growth is an important factor relative to timing. The optimal period for infection during this study was during rapid shoot elongation before the needles have grown more than one centimeter. Unless all seedlings are inoculated during this optimal infection period, there is the possibility of escape due to phenological timing. It is also advantageous to have the healthiest seedlings possible to insure that infection and death will be due to *S. sapinea* and not other causes.

Wang et al. (1985) described 2 strains of *S. sapinea*, types A and B, which differ in culture morphology, growth rate, spore characteristics, and virulence. Type A was able to infect unwounded tissue but type B required wounded tissue to successfully invade (Palmer et al., 1987; Wang et al., 1985). Positive identification of either type was difficult using gross cultural characteristics, because they can differ when the fungus is grown on different media and in different environments (Wang et al., 1985). The most relible method to determine type was to examine the spore surface with a scanning electron microscope; type A had a smooth spore surface and type B a pitted surface. Due to differing virulence between the 2 strains, Palmer et al. (1987) suggested identifying strains when reporting results.

No evidence was found in this study to support the notion of *S. sapinea* strains differing by spore type. Swart and Wingfield (1991) also found no evidence that local isolates in South Africa could be classified into distinct groups based on growth rate of cultures and virulence of isolates. They proposed that *S. sapinea* is a highly variable species with virulence better explained as a continuum rather than differing among distinct population types or strains. Results of this study are consistent with their conclusion.

## Conclusion

It is possible to artifically inoculate *S. sapinea* to screen for resistance in Scotch pine seedlings. The next step in developing a resistance screening method should be to confirm the validity of this technique while determining whether using healthier, better established seedlings exposed to higher spore concentrations would improve the results. If seedlings had been planted the season before screening, they would have been well established and healthier when the inoculations were taking place.

Since the date of inoculation itself was not an important factor, the inoculations should be timed according to stage of growth. Varietal or family differences in susceptibility can be detected best when the seedlings are inoculated during shoot elongation and before much growth of the needles. Choosing several inoculation dates during the optimal susceptibility period of about 3 weeks would allow detection of different patterns of resistance among varieties.

Spore concentration was an important factor that should be examined further. Finding a suitable method to produce higher infection levels would be very helpful in further developing this screening process. The use of low nutrient media, in combination with high humidity and a 24 hour photoperiod, produced adequate numbers of pycnidia in culture. Spore concentrations could be adjusted by centrifuging the spores at low speeds after extraction, resuspending them in water, and adjusting to desired spore concentrations by dilution. Applying greater volumes of inoculum to the seedlings or using a more effective method of application are also possibilities for increasing the spore load.

The varietal differences in susceptibility suggest that genetic improvements in resistance are possible. The inoculation method may be employed to screen for resistant individuals and families for use in breeding and in seed orchards.

## Literature

Brookhouser, L. W. and Peterson, G. W.: Infection of Austrian, Scotch, and ponderosa pines by *Diploida pinea*. Phytopathology **61**: 409—414 (1971). — Burdon, R. D., Currie, D. and Chou, C. K. S.: Responses to inoculation with *Diploida pinea* in progenies of apparently resistant trees of *Pinus radiata*. Australian Plant Pathology **11**(4): 37—39 (1982). — Chou, C. K. S.: A shoot dieback in *Pinus radiata* caused by *Diploida pinea*. I. Symptoms, disease development, and isolation of pathogen. New Zealand Journal of Forestry Science **6**(1): 72—79 (1976a). — Chou, C. K. S.: A shoot dieback in *Pinus radiata* caused by *Diploida pinea*. II. Inoculation studies. New Zealand Journal of Forestry Science **6**(3): 409—420 (1976b). — Chou, C. K. S.: *Diploida pinea* infection of *Pinus radiata* seedlings: effect of temperature and shoot wetness duration. New Zealand Journal of Forestry Science **12**(3): 425—437 (1982a). — Chou, C. K. S.: Susceptibility of *Pinus radiata* seedlings to infection by *Diploida pinea* as affected by pre-inoculation conditions. New Zealand Journal of Forestry Science **12**(3): 438—441 (1982b). — Gregory, P. H.: Microbiology of the atmosphere. 2nd ed. Leonard Hill Books (1973). — Haddow, W. R. and Newman, F. S.: A disease of the Scotch pine (*Pinus sylvestris* L.) caused by *Diploida pinea* Kickx. associated with the pine spittlebug (*Aphrophora parallela* Say.). Transactions Royal Canadian Institute **24**(1): 1—18 (1942). — Ingold, C. T.: Fungal spores: their liberation and dispersal. Clarendon Press (1971). — Palmer, M. A., Stewart, E. L. and Wingfield, M. J.: Variation among isolates of *Sphaeropsis sapinea* in the north central United States. Phytopathology **77**: 944—948 (1987). — Peterson, G. W.: Infection, epidemiology, and control of *Diploida* blight of Austrian, ponderosa,

and Scotch pines. Phytopathology 67: 511—514 (1977). —— PUNITHALINGHAM, E. and WATERSON, J. M.: *Diploida pinea*. C. M. I. Descriptions of pathogenic fungi and bacteria. No. 273. (1970). —— SINCLAIR, W. A., LYON, H. H. and JOHNSON, W. T.: Diseases of trees and shrubs. Cornell University Press. 574 p. (1987). —— SWART, W. J. and WINGFIELD, M. J.: Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. Plant Disease 75(8): 761—766 (1991). —— SWART, W. J., WINGFIELD, M. J. and KNOX-DAVIES, P. S.: Relative susceptibilities to *Sphaeropsis sapinea* of six *Pinus* sp. cultivated in South Africa. European Journal of Forest Pathology 18: 184—189 (1988). —— WANG, C.-G., BLANCHETTE, R. A., JACKSON, W. A. and PALMER, M. A.: Differences in conidial morphology among isolates of *Sphaeropsis sapinea*. Plant Disease 69 838—841 (1985). —— WRIGHT, J. W., LEMMIEN, W. A., BRIGHT, J. N., DAY, M. W. and SAJDAK, R. L.: Scotch pine varieties for Christmas tree and forest planting in Michigan. Michigan State University, Agricultural Experiment Station Research Report 293. 15pp. (1976). —— ZWOLINSKI, J. B., SWART, W. J. and WINGFIELD, M. J.: Intensity of dieback induced by *Sphaeropsis sapinea* in relation to site conditions. European Journal of Forest Pathology 20: 167—174 (1990)

# Effects of Sib Mating on Cone and Seed Traits in Coastal Douglas-Fir

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**Summary**

Outcross, half-sib and full-sib, and self matings were made in an Oregon seedling seed orchard in 1987 and 1989. Cone and seed traits other than fertility, but including germination, were almost exclusively under maternal influence. The relations between 3 measures of fertility and inbreeding coefficient (F) were linear or predominantly linear. Our results were very similar to those previously reported by WOODS and HEAMAN (1989), and combining their results with ours gave 40.2, 30.9, 23.2, and 1.15 filled seeds per cone after cross, half-sib, full-sib, and self pollination, respectively. Selfing appeared to reduce seed set more in the orchards than in natural stands. Assuming linear relations between F and fertility and between F and vigor, it was shown that partial selfing between zero and 50% has a greater impact than partial sibbing on seed set; but that partial sibbing, particularly full-sibbing, has the greater impact on population vigor and wood production.

*Key words: Pseudosuga menziesii*, seed orchard, inbreeding, inbreeding depression, fertility, germination, mixed mating.

*FDC:* 165.41; 181.5; 232.311.3; 174.7 *Pseudotsuga menziesii*.

## Introduction

Many conifer seed orchards in the Pacific Northwest have been established with full- and half-sib seedling progenies, sometimes in conjunction with clonal material from parents of the progenies (WHEAT and BORDELON, no date). Having related individuals in the same orchard provides opportunity for both selfing and several types of lower intensity inbreeding. In this paper, we report on the effects of self, full-sib, half-sib, unrelated, and wind pollination on cone size and seed traits, including germination, using trees in a Douglas-fir seedling orchard in Oregon. Results from 2 years of pollination are compared with those reported by WOODS and HEAMAN (1989), who contrasted several levels of inbreeding with outcrossing in their effect on seed production in a Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) full-sibling factorial progeny test and in clone banks for the factorial on southern Vancouver Island.

## Materials and Methods

All pollinations were made in the Vernonia block of the J. E. Schroeder Cooperative Seed Orchard, managed by the Oregon State Department of Forestry and located in the Willamette Valley about 25 km north of Salem, Oregon, at an elevation of about 50 m. Parent trees of the Vernonia block progenies are native at low elevation (250 m to 500 m) in the northwest corner of the Oregon Coast Range. The block contains full-sib families from biparental matings made in the natural stands. In a few cases, more than 1 family has a common parent.

*Trial 1* pollinations were made in 1987 in the oldest portion of the Vernonia block. Trees were 15 years old and had a mean height of about 12 m. Pollinations were made on a total of 20 seed trees representing 12 full-sib families; i.e., trees were replicated within 8 of the families. Self (inbreeding coefficient, F = 0.5), full-sib (F = 0.25), and outcross polymix (F = 0) pollens were applied to each seed tree, and half-sib (F = 0.125) pollens were applied to 3 trees. The outcross polymix included equal volumes of pollen from 3 to 5 families, unrelated to the seed trees, but in the same orchard block. Several mixes were used in the course of the pollinations.

Isolation bags were applied to branch ends in the upper parts of crowns shortly before floral bud burst. Pollen was obtained from microsporangiate strobili on detached twigs after drying at about 25 °C in a low-humidity room. For pollination, bags were removed, pollen was poured from a small vial onto receptive female strobili (OWENS et al., 1981), and isolation bags were immediately reinstalled on branches. If any female strobili were not receptive at the time of first pollination, the bag was so marked and the flowers within it repollinated 3 to 4 days later. Only fresh pollen was applied. All work was done from a hydraulic lift. To control insect damage to cones and seeds, isolation bags were left in place until cone harvest.

Cones were collected in late summer shortly before scales started to flare. Five undamaged cones from each mating, if available, were bagged separately and set aside for dissection and estimation of seed production. (Seeds were extracted from other cones but were not used in determining seed production traits.). Sample cones were individually sealed in small kraft bags, initially dried in an open air shed, and given final drying at 20 °C to 25 °C in a heated room with dehumidifier. Cones were then taken apart by hand and, within the presumed fertile part of each cone, undeveloped flat and normal-appearing round seeds were counted. Round seeds were x-rayed and filled and empty seeds counted. Total filled seeds from each cone were weighed to the nearest mg.

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