Heritability and Gain of Reduced Spotting vs. Blister Rust on Western White Pine in British Columbia, Canada

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Abstract

Analyses of rust spots per seedling and cankerling percent-ages of 215 families of western white pine in British Columbia, inoculated under controlled conditions in variable numbers in 4 inoculation years, indicate that spot frequency per seedling is under genetic control. For families with heritability estimates ranging from 18.2% to 86.6%, averaging 77.3% for the years for which adequate families for selection were screened using 2-year-old seedlings. Confidence limits ranged from 12% to 163%.

orchard should double the gain to about 100%. Gains by using open-pollination progeny from the "selected" candidates over seedlings from unselected candidates could reach about 27.5%.

**Key words:** *Pinus monticola*, *Cronartium ribicola*, rust resistance, selection, disease resistance.

**FDC:** 165.53; 165.62; 443; 172.8 *Cronartium ribicola*; 147.7 *Pinus monticola*; (711).

**Introduction**

Western white pine (*Pinus monticola* D. DON) is a vigorous pioneer species native to southern British Columbia (B. C.) and adjacent U. S. states that produces clear, straight stems and high-value lumber. It occurs in 2 disjunct areas: the Coast to approximately 51°N latitude, and the Interior "wet belt" Columbian forests to approximately 53°N latitude: Rowe’s Coast and Columbia zones (Rowe, 1972). Since its introduction at Vancouver about 1910 (Gussow, 1923; Hoff, 1988), white pine blister rust (*Cronartium ribicola* J. C. Fisch.) has infected western white pine throughout its range, killing both mature and immature trees, and suspending efforts to regenerate it artificially. Indeed, from about 1960 to 1984, western white pine was not tallied during restocking surveys on Crown Land in British Columbia because of the high rust-caused mortality found throughout the range (Muir, 1988).

Early efforts to combat this rust included suppression of the alternate host, *Ribes* species (Franc, 1988) and selection and screening of individuals displaying no, or low, rust infection in the forest, so that the better selections could be used as seed parents of planting stock. During the 1950s, Porter (1960) selected and grafted phenotypically-resistant B. C. white pines and subjected the ramets to annual inoculation in an outdoor "Ribes garden". The better clones, plus screened material from the U. S. Forest Service’s testing program for Region One (Rocky Mountains), were planted on a number of sites over time.

After suspension of the B. C. program in 1960, the prospects of resurrecting western white pine as a species worthy of management in the province were evaluated, starting in 1979. A Memorandum of Understanding was signed in 1983 by the British Columbia Ministry of Forests and the Canadian Forest Service "To provide for ... a program for the production, testing and replication in seed orchards of western white pine breeds having increased levels of resistance to the blister rust ...". The first inoculations were attempted in 1986. This article summarizes procedures followed, analyses results of rust spots per seedling by family from the first 4 inoculation years, and estimates gain in reduced infection intensity via seed collections *in situ* and from future seed orchards.

**Materials and Methods**

**Parent trees**

Candidate trees (PTs) selected for screening were from 20 years to 35 years old, i.e. had regenerated naturally in the presence of the blister rust, and displayed field "resistance" to the rust: the majority was canker free, while a few displayed a "tolerant" reaction in the stem (Hoff, 1984). Open-pollinated seeds were sown in family lots in styroblocks, then transplanted prior to the second year into randomised 9-tree rows ("plots") in 45-cell styroblocks. Each candidate tree (family) was represented by up to 90 seedlings in 10 replicates, except in 1986, when 3 to 16 replicates per family were assessed. Starting in 1988, live primary leaves were removed by hand before inoculation, since they are very highly susceptible (Hunt, 1991).

**Inoculation**

"Inoculation" was conducted under cool, moist conditions (temperatures 10°C to 18°C) in a large cold-storage unit. Infected *Ribes* leaves from a number of cultivated and natural groves, each inoculated by us using a different Coastal source of aeciospores, were mixed and placed on mesh racks about 80 cm above the seedling canopy. Casting of the haploid basidiospores was monitored on slides placed regularly throughout the seedling blocks. Once the average of 3000 spores per cm² was reached, the stock was shifted away from the *Ribes* leaves to spend another 48 h in the chamber, so that spore germination and penetration of leaves could occur. Light was supplied intermittently to assist in stomatal opening during the latter 48 h. Stock then was returned to a nursery until spot counting the following spring. Rust development was recorded each autumn or winter thereafter until the time for required for expression of each reaction type has passed – up to an additional 4 years in the case of slow-developing cankers (Hoff and McDonald, 1980).

**Families**

The number of families tested and experimental design are summarised by inoculation year in table 1. Parents screened

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**Table 1. – Summary of families screened and experimental design by inoculation year.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Families</th>
<th>Replicates per family</th>
<th>Mean Seedlings /rep/ family</th>
<th>Experimental Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986/7</td>
<td>19</td>
<td>3-16</td>
<td>8.2</td>
<td>All blocks randomised in 1 chamber</td>
</tr>
<tr>
<td>1987</td>
<td>65</td>
<td>10</td>
<td>9</td>
<td>All blocks randomised between 2 chambers</td>
</tr>
<tr>
<td>1988</td>
<td>64</td>
<td>10</td>
<td>9</td>
<td>All blocks randomised between 2 chambers</td>
</tr>
<tr>
<td>1989</td>
<td>72</td>
<td>10</td>
<td>9</td>
<td>All families randomised equally in 2 sets - 1 set per chamber</td>
</tr>
</tbody>
</table>
were chosen from principally the Coastal zone, to produce a seed orchard. More interior and U.S. parents were screened in 1989 due to lack of seed from Coastal parents. Although inoculation chamber was not large enough to accommodate all stock in a single inoculation chamberful (run) the randomisation of families to styroblocks for the 1986/1987 to 1988 inoculations was not restrained. Thus, families might have been represented by different numbers of plots per run in those inoculations. For the 1989 inoculation, progeny per family were assigned equally to 2 sets including all families randomised within set. Each set was inoculated in a separate run.

1986/1987

Due to inadequate infection by the spring of 1987, all 1986 stock was re-inoculated in 1987. One family was from a heavily cankered tree (PT 74) used here and in subsequent years as a control.

1988

Thirty-two of the 64 PTs screened are from the B. C. Interior. Four of the families were from new seed collections from PTs whose progeny were inoculated in 1986/1987.

1989

The 72 PTs screened included 27 from the B. C. Interior, 20 from the U. S. Coast and 10 from the U. S. Interior. Four progenies were tested earlier, including 2 from PT 48.

1990

Data from this inoculation, involving 149 PTs, are not discussed because all were tested using 30 or fewer seedlings in order to screen out highly susceptible families. However, 2 bulked seedlots (collected from many trees in separate natural stands without regard to blister rust “resistance”) were included as controls with 107 or 120 seedlings. Their mean-spot percentile was calculated and used as a presumed level of susceptibility by seedlings grown from bulked seed collections, had they been tested in the preceding inoculation years. That estimate was compared to the value predicted, using spots heritability (see below), for the selected families in each year and the difference was expressed as percentage in reduction of mean spots via collecting open-pollinated seeds from the selected candidates in situ.

Inoculations

Sample size

Ninety seedlings per family, arranged in 9-seedling rows in styroblocks containing 45 cavities of 328 cc, were the basic testing unit per candidate. Seedlings were transplanted into this pattern before flushing the year of inoculation, following a randomisation pattern derived from SAS.

Spot counting

Spots were counted in May, 8 to 9 months after inoculation. Generally, all spots were counted for each seedling, but the following limits were placed on the tally per seedling to speed the work after ascertaining that they represented the higher counts for seedlings in highly spotted blocks: 1986/1987 and 1987 – all spots above 50 were recorded as “51”; 1989 – all spots above 98 were recorded as “99”; 1988 – spots were recorded as "large" (spot length greater than leaf length), or "small" (spot length less than leaf length), with a maximum of 50 in each category. Thus, the limit changes might affect both means and variances among years. Seedlings with primary needles were eliminated from the data sets as primary needles are more susceptible than secondary needles (Hunt, 1991).

Canker development

All seedlings were examined in the fall or winter 14 months to 18 months after inoculation. Seedlings displaying stem discoloration or swelling were considered cankered.

Data analysis

Replicate mean spots per family, and replicate mean of \( \sqrt{\text{spots}} + 0.325 \) (Zar, 1984, p. 241) per family, were the basic statistics analysed in order to avoid problems with skewed variances due to zero values. The assumption of normality was violated, but this transformation came closest to reaching normality. Also, data for the "control" tree (PT 74) were first included, then eliminated from analysis of family heritability because it is an atypical (heavily cankered) tree and because it was over represented in the data (from 25 to 40 replicates per inoculation for tests in 1987 to 1989). Family heritabilities were calculated as 4 times the covariance of half sibs, based on replicate means per family of both raw and transformed data. SAS procedures for unbalanced data sets were employed, treating PT as a fixed effect. Variance components (Type 1) were obtained from the SAS Variance Component procedure for both mean and transformed data. Confidence limits on heritability estimates were computed using the method in Beck (1984) for unequal numbers of progeny per parent. Pearson product-moment correlations were calculated by SAS ‘PROC CORR’ procedure.

Although heritability estimates should be based on a random sample of parents in a species, estimates here are derived from selected parents displaying phenotypical "resistance" to blister rust. Gain estimates are based on heritabilities applied to such parents and are compared to heritabilities and gains calculated by others using similar criteria in programs of selection against white pine blister rust.

The estimate of gain by selection of the “low-spot” families (those ranked above or equal to the control family) was calculated from the following equation:

\[
\text{Predicted spots} = \text{Mean all} - H_{FAM} \left( \text{Mean all} - \text{Mean select} \right)
\]

\[H_{FAM}\] is estimated family heritability by year.

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![Image](https://via.placeholder.com/150)

**Figure 1.** Frequency distribution of families by mean spots per seedling class for 1986/1987 inoculation. “Mean” indicates location of mean of all families. “C” indicates mean spots for “control” tree PT 74.

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Since no unselected parents were tested in the inoculations analysed here, estimates of the mean spots for "standard" seedlots (representing collections from unselected parents in natural stands) were obtained from two such seedlots in the 1990 inoculation, in the following way: the mean spots percentile of these seedlots was calculated (SOKAL and ROLHF, 1981) and this value was used to estimate the mean spots corresponding to that percentile for each inoculation from 1987 to 1989. This value of mean spots per seedling was entered in the formula to estimate gain.

Results

Inoculation success

Infection percentage of seedlings surviving at the time of spot counting was 18.9%, 84.6%, 78.9% and 97.0% for the 1986/1987, 1987, 1988 and 1989 inoculations, respectively.

Two seedling-vigour classes from the 1987 and 1988 inoculations were analysed for correspondence with mean spots. Only "small" seedlings (conspicuously shorter than their siblings in the same seedling block), but not "weak" seedlings (conspicuously frailer than their siblings in the same seedling block) showed reduced spot numbers, and thus were eliminated from further analyses.

Family mean spots per seedling and parental ranks

Figures 1 to 4 present frequency distributions of parents among classes of mean spots per seedling by inoculation year. The mean of spots per seedling by family is indicated on figures 1 to 4 and listed by inoculation year in table 2. The distributions within year are approximately normal, except that there is a cluster of low-spot families in the vicinity of the "control" family in the 1987, 1988 and 1989 inoculations. When the separate inoculation runs from 1989 were analysed, similar patterns of groupings appeared, but the means were dispersed more widely. Correlation of family rank between the 2 1989 inoculation runs was 0.495 (P = 0.0001, n = 72).

Ranking varied for PTs tested using more than one seedlot or re-testing the same seedlot (Table 2 and Figures). The control tree (PT 74) ranked near the mean from one seedlot (1986/1987 inoculation) and well above (fewer spots) the mean (spot percentiles from 10% to 20%) in subsequent years, when a different seedlot was tested. PTs 48 and 62 also had similar rankings when the same seedlot was tested in different years. Separate seed collections from a PT ranked similarly in 3 cases (PTs 49, 61 and 62), differed in one case (PT 60) and ranked inconsistently in one (PT 48).

No significant difference was found by SAS GLM between the mean spots per seedling of 29 B. C. Coastal (16.6 spots) vs. 32 B. C. Interior (19.4 spots) families inoculated in 1988, whereas the mean of 14 B. C. Coastal families (46.3 spots) was significantly lower than those of 27 B. C. Interior (57.4 spots) and 10 U. S. Interior (55.9 spots) families inoculated in 1989.

Table 2. – Parent-tree rank percentile for parents tested more than once, based on family mean spots per seedling, by inoculation year.

<table>
<thead>
<tr>
<th>Parent Tree No.</th>
<th>Seedlot</th>
<th>Inoculation Year</th>
<th>1986/7</th>
<th>1987</th>
<th>1988</th>
<th>1989</th>
<th>Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>74a</td>
<td></td>
<td>58</td>
<td>11</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>74b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>48a</td>
<td></td>
<td>25</td>
<td>18</td>
<td>2</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>49a</td>
<td></td>
<td>33</td>
<td></td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>60a</td>
<td></td>
<td>79</td>
<td></td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>61a</td>
<td></td>
<td>45</td>
<td></td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>61b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>62a</td>
<td></td>
<td>8</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>62b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) Percentile value (from 1 to 100) of family mean spots in inoculation year (SOKAL and ROLHF, 1981).
The mean of the 20 U. S. Coastal families (48.4 spots) did not differ significantly from that of any other source.

Family spotting heritability 1986/1987 to 1989

Including data from the control family increased the heritability value 2% to 9%, except for the 1986/1987 inoculation, where the increase averaged 20% (Table 3). Transforming the data for analysis increased heritability values for 1987 and 1988, but lowered them for 1986/1987 and 1989 (Table 3).

Mean family heritability of untransformed data was estimated as 62.5% (range 18% to 87%) over the 4 years of inoculations (Table 3).

The separate inoculation runs in 1989, with mean spots per seedling averaging 48.6 and 53.5, produced heritability values of 78% and 112%. Confidence limits about heritability values were very broad, often exceeding 0 (minus values) or 100%.

Estimated gain in resistance 1987 to 1989

The estimated change in mean spots per seedlings by collecting seeds in situ from all candidates, or only those top-ranked, are presented by inoculation year in Table 4. Heritability used was from transformed data, excluding the control family (Table 3). Results from 1986/1987 are excluded because of the low number of families tested and the stock being 3 years old when inoculated, thus more difficult to infect (Hunt, 1991).

The spots percentile of the 2 bulk seedlots inoculated in 1990 averaged 80. This percentile corresponded in the 1987, 1988 and 1989 inoculation results to 22.1, 24.3 and 64.2 spots per

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**Table 3.** Summary of family spot-frequency heritability analyses.

<table>
<thead>
<tr>
<th>Inoculation year</th>
<th>Control seedlot included</th>
<th>Control seedlot excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n2</td>
<td>Data form</td>
</tr>
<tr>
<td></td>
<td>Mean spots</td>
<td>Transformed</td>
</tr>
<tr>
<td>1986-7</td>
<td>183</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td>Limits</td>
<td>-8.4-81.4</td>
</tr>
<tr>
<td>1987</td>
<td>675</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Limits</td>
<td>40.9-86.7</td>
</tr>
<tr>
<td>1988</td>
<td>685</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>Limits</td>
<td>63.4-133.2</td>
</tr>
<tr>
<td>1989</td>
<td>720</td>
<td>85.8</td>
</tr>
<tr>
<td></td>
<td>Limits</td>
<td>56.8-123.1</td>
</tr>
<tr>
<td>SET 1</td>
<td>360</td>
<td>78.3</td>
</tr>
<tr>
<td></td>
<td>Limits</td>
<td>39.6-126.9</td>
</tr>
<tr>
<td>SET 2</td>
<td>360</td>
<td>115.0</td>
</tr>
<tr>
<td></td>
<td>Limits</td>
<td>72.7-165.4</td>
</tr>
</tbody>
</table>

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**Table 4.** Estimated percentage genetic gain via screened candidates (PTs), selected (low-spot) candidates vs. "bulk" collections by inoculation year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Candidates</th>
<th>Mean spots per seedling</th>
<th>Gain %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Screened</td>
<td>Selected</td>
<td>%</td>
</tr>
<tr>
<td>1987</td>
<td>65</td>
<td>7</td>
<td>76.7</td>
</tr>
<tr>
<td>1988</td>
<td>64</td>
<td>9</td>
<td>97.8</td>
</tr>
<tr>
<td>1989</td>
<td>72</td>
<td>7</td>
<td>67.7</td>
</tr>
<tr>
<td>Mean 1987-1989</td>
<td>6.6</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.5</td>
</tr>
</tbody>
</table>

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1) SAS Type 1.
2) Number of plots.
3) Transformed by \sqrt{spots} + \sqrt{spots} + 0.325 and averaged by family in replicate.
The mean reduction in predicted mean spots by phenotypic selection of candidates averages 24.5%, whereas the gain from collecting open-pollinated seed from the "selected" low-spot candidates averages 52% for 1987 to 1989, inclusive (Table 4).

The impact of family rust spotting on stem cankering 18 months after the 1988 inoculation is shown in Figure 5. There is a highly significant (P = 0.0001) trend of increased cankering with increasing family spotting, permitting early culling of the high-spot families. However, 8 low-spot families (47, 48, 62, 112, 126, 128, 180 and 239) ranged between 22% and 75% in cankering. The control family was only slightly below average in percent cankered (Figure 5).

![Figure 5](image.png)

**Figure 5.** – Trend of family mean number of spots per seedling and cankering percentage 18 months after the 1988 inoculation.

### Discussion

Percentage of seedlings spotted in 1987 (85%) and 1988 (79%) was greater than for the control lots (77%) reported by Bingham (1972); the spotting level in 1989 (97%) approached the "... more than 99%" reported by Hoff (1988) for a 1966 inoculation. Thus, our inoculation success is similar to that reported by experienced workers in Idaho. Within year, the range of means of spots per seedling among families differed by multiples of 3 (1989) to 20 (1988) (Figures 1 to 4); 1988 gave the highest heritability value (86.6%), while 1986/1987 was lowest (18.2%).

Following the modification of inoculation design from complete randomisation to 2 sets in 1989 and tripling of infection intensity from about 16 spots per seedling in 1987 and 1988 to 51 (Table 2), heritability remained high (Table 3). Differences in family heritability were obtained from the separate inoculation runs in 1989. However, the reasonably-consistent rank of the same seedlots on retesting and the generally-similar rank of different seedlots from the same parents (Table 2) indicate that the least-susceptible parents have been identified.

The stability of families from the highly cankered control tree and from other trees tested more than once, perhaps using repeated seed collections, confirms that spot number is under genetic control, and that one seed collection per parent and one successful inoculation may be sufficient for reliable ranking of a candidate. The mean heritability for 1987 to 1989 (77.3%) is comparable to values computed for the percentage of healthy seedlings in controlled-pollination families (68.8% Bingham et al., 1960, and a mean of 85.1% for 3 years Bingham et al., 1969). However, heritability of spots per meter of leaf in similar material screened in Idaho was 46% (Hoff and McDonald, 1980). These differences in heritability may reflect more the differences in inoculation conditions than among family means or in efficacy of field selection of candidates.

Our heritability estimates are derived from open-pollination families from selected trees, perhaps involving many pollen parents per family (El-Kassaby et al., 1987). Although the heritabilities obtained properly apply to only these tested trees, we have used the values to estimate the gain over bulked seed collections from unselected parents, which are currently used in B. C. (Table 4).

Although variability in infection intensity within the test may be a major factor in heritability estimates, part of the difference in heritability values between our tests vs. those of Hoff and McDonald (1980) may be due to the multiplier of four applied to our covariance of half sibs to estimate heritability. This multiplier assumes that each seedling is the offspring of a different pollen parent and that all pollen parents are unrelated. This is surely untrue in nearly all natural plant populations, leading to mating among relatives (Namkoong, 1966) and inflated estimates of heritability (Squillace, 1974).

However, despite using a multiple of only 3, Mullin et al. (1995) estimated some heritabilities exceeding 100% in black spruce tests. This illustrates the sensitivity of heritability estimates to test material and environments.

Barnes et al. (1962) found "... a moderate to strong discrimination against self-pollen "..." in most of the trees tested and predicted "... a relatively small amount of germinable seed resulting from self-pollinations in a seed orchard." Squillace and Bingham (1958) felt that outcrossing would exceed selfing in most trees under open pollination in natural stands. Variation in the outcrossing rate among parents in the stand containing PT 48, and different outcrossing rates by seed parents among seed years, were reported by El-Kassaby et al. (1993). Some trees were consistent "inbreeders", while others were consistent "outcrossers". Inbreeding was assumed due to consanguineous mating.

The bias due to the situation here, where seed parents are likely uncorrelated and natural-selfing level is presumed low (5% or less), is approximately 10% upward, which still leaves our estimates for 1987 to 1989 above those of Hoff and McDonald (1980). This may be due partly to differences in susceptibility to rust among the populations represented, as suggested by the differences between Coastal and Interior candidates found here and in field trials (Hunt and Meagher, 1989; Hunt, 1994). Assuming that some loci and alleles are common among parents, average gain in reduced spots from a clonal seed orchard of the selected parents should double, i.e. up to a mean of 82%. However, Hoff and McDonald (1980) suggest that a single non-dominant gene may be involved, so that gain from a clonal seed orchard may be less.

The stability in rank of parents tested more than once and estimates of heritability obtained indicate that the low-spot parents have been identified and that immediate genetic gain against blister rust can be obtained by collecting seed from the lowest-spotting trees in situ, as found also by Bingham et al. (1969). Although the selection differentials in mean spots appear high, they are below one standard deviation (0.8 in 1987 and 1988, and 0.8 in set 1 of 1989 and all 1989 sets pooled) for all but set 2 in 1989, when it was 1.24 standard deviations. Thus, reasonably mild selection of the least-susceptible families might yield useful gain.
The high percentile ranking (high susceptibility) of bulk seedlots from untested white pine trees to blister canker caused by the fungus Cytospora californica were found to have a significant effect on the survival of young seedlings. The authors thank J. Bramble and G. Jensen for technical support, C. Simmons for statistical advice and Y. A. El-Kassaby and an anonymous reviewer for careful reviews and helpful comments.

References