Forest pathology

# Relationship between early family-selection traits and natural blister rust cankering in western white pine families

**R.S. Hunt**

**Abstract**: Blister rust (*Cronartium ribicola*) cankering incidence was compared among half-sib families of both artificially and naturally inoculated western white pine (*Pinus monticola*). In the first test, artificially inoculated seedlings of six families had their individual infection spots tallied and any infected needles were removed before cankers developed. These healthy seedlings were classified as high or low spotters and were planted in row plots with four high spotters paired with four low spotters. After 7 years, 2 pairs had equal cankers, 12 had more rust on the high spotters, and 12 had more rust on the low spotters. The mean number of cankers per tree was 22 and 23 for high and low spotters, respectively. In the second test, 203 artificially inoculated western white pine families were ranked for their relative susceptibility to blister rust based on (*i*) family mean counts of individual infection spots per seedling (spotting) and (*ii*) the presence of only small cankers (slow canker growth resistance). These results were compared with the natural cankering incidence of the same families in eight plantations. There were few significant differences in spotting incidence among inoculated families. The range in field cankering in the test plantations (15–63 families each) was 22–88%. Seven plantations, including two with families significantly different in infection-spotting incidence, lacked a significant correlation (Spearman, $P < 0.05$) between percentage field cankering and spotting incidence from inoculations. Although one plantation had a significant correlation ($r_s = 0.64$; $P < 0.005$) between spotting incidence from inoculation and cankering in a plantation, there was no overall trend across plantations. Incidence of slow canker growth resistance from artificial inoculation ranged from 0 to 18%. There was a significant positive correlation between field cankering and slow canker growth resistance, as determined by inoculation only in three of eight plantations. However, when the families were placed into slow canker growth classes, there was a trend of reduced cankering with increasing class of slow canker growth resistance (mean Spearman's $r_s$ value = 0.87 ($P < 0.01$) across all plantations). These studies indicate that selection on the basis of slow canker growth resistance resulted in less cankering in plantations than selection on the basis of reduced infection spotting.

*Key words*: white pine blister rust, *Cronartium ribicola*, *Pinus monticola*.

**Résumé** : La fréquence des chancres de la rouille vésiculeuse du pin blanc (*Cronartium ribicola*) fut comparée entre des familles à demi-apparentées du pin argenté (*Pinus monticola*) inoculées artificiellement et naturellement. Lors du premier test, les tachetures individuelles des semis artificiellement inoculés de six familles furent inventoriées, puis les aiguilles infectées furent enlevées avant que les chancres ne se développent. Ces semis en santé furent classés comme très ou peu sujets aux tachetures et furent plantés en parcelles disposées en rangées, quatre semis très sujets aux tachetures étant regroupés par paire avec quatre semis peu sujets aux tachetures. Après 7 ans, 2 paires présentaient la même quantité de chancres, 12 avaient plus de rouille sur les individus très sujets aux tachetures et 12 en avaient plus sur les individus moins sujets aux tachetures. Le nombre moyen de chancres par arbre était de 22 et 23 pour les individus très sujets et ceux peu sujets aux tachetures, respectivement. Dans le second test, 203 familles de pin argenté artificiellement inoculées furent classées en fonction de leur sensibilité à la rouille vésiculeuse en fonction (*i*) du nombre moyen de tachetures par semis par famille et (*ii*) de la présence de petits chancres seulement (résistance de type « développement lent des chancres »). Ces résultats furent comparés à la fréquence des chancres naturels dans les mêmes familles dans huit plantations. Il y avait peu de différences significatives dans la fréquence des tachetures entre les familles. La fourchette de fréquences de chancres dans les plantations du test (15–63 familles chacune) était de 22 à 88%. Sept plantations, y compris deux plantations avec des familles significativement différentes au chapitre de la fréquence des tachetures, ne montraient pas de corrélation significative (Spearman, $P < 0,05$) entre la fréquence de chancres naturels et celle des tachetures dues aux inoculations. Quoiqu'une plantation montrait une corrélation

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**R.S. Hunt.** Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria BC V8Z 1M5, Canada (e-mail: rhunt@pfc.forestry.ca).

significative ($r_s$ = 0,64; $P$ < 0,005) entre la fréquence des tachetures dues à l'inoculation et le développement de chancres en plantation, il n'y avait pas de tendance générale, toutes plantations confondues. La fréquence de la résistance de type « développement lent des chancres » par suite de l'inoculation s'échelonna entre 0 et 18%. Il y avait une corrélation positive significative entre le développement de chancres au champ et la résistance de type « développement lent des chancres » telle que déterminée dans des inoculations de seulement trois des huit plantations. Cependant, lorsque les familles furent placées dans des classes de « développement lent des chancres », il y a eu une tendance à la diminution du développement de chancres à mesure qu'augmentait la classe de résistance « développement lent des chancres » (valeur moyenne du $r_s$ de Spearman 0,87 ($P$ < 0,01), toutes plantations confondues). Ces études indiquent que la sélection des familles pour la résistance « développement lent des chancres » a abouti à moins de chancres dans les plantations que la sélection pour moins de tachetures dues à l'infection.

*Mots clés* : rouille vésiculeuse du pin blanc, *Cronartium ribicola*, *Pinus monticola*.

## Introduction

Western white pine (*Pinus monticola* D. Don) is a desirable tree for reforestation in the Pacific Northwest, because it has high stumpage values and resistance to root disease (Muir and Hunt 2000; Nelson and Sturrock 1993). The introduced stem rust *Cronartium ribicola* J.C. Fisch. may cause mortality in excess of 95% in unmanaged plantations (Hunt and Meagher 1989). The inoculum for this infection generally comes from outside of the stand, i.e., from the alternate host *Ribes* spp. growing in wet areas or sometimes in open areas created by root disease or partial cuts (Mielke et al. 1937; Buckland 1946; Hopkins 1974; Hunt 2000). Removal of the lower branches from white pine can save plantations where the incidence of *Ribes* spp. is low (Hunt 1998, 2000). Resistant stock needs to be employed on high-hazard sites (Hagle et al. 1989) and resistance can be used to alleviate the costs of pruning on low-hazard sites.

In British Columbia, to screen seedlings for resistance, annual inoculations were made in an inoculation chamber from 1986 to 1995. Selection was based on two non-race-specific traits: few individual infection spots per seedling (Meagher and Hunt 1996) (= reduced needle-lesion frequency; Hoff and McDonald 1980) and abnormally small cankers (i.e., slow canker growth) (Hunt 1997). Fewer infection spots generally means fewer cankers at 16 to 18 months post inoculation (Hunt and Jensen 1999); thus, a family is considered more resistant if its mean spot frequency is less than that of another family. At the U.S. Forest Service Dorena Tree Improvement Centre in Oregon, the goal is to reduce infection spotting and hopefully cankering as well by 10 fold (Samman and Theisen 1980). In B.C., the initial goal was to cull highly spotted families (Hunt and Meagher 1993) and retain the top 50 least-spotted families out of 300 to be inoculated (Meagher et al. 1990). Fifty genetically unrelated individuals are believed to be a good genetic base on which to build a seed orchard (Hunt et al. 1985). The incidence of slow canker growth is generally low within families and it is used as a progeny-selection trait (Hunt 1997).

The objective of this study was to evaluate half-sib families for the relationship between field canker incidence in western white pine plantations and the incidence of two resistance screening traits: reduced infection spotting and slow canker growth.

## Materials and methods

### Experiment 1

To compare field cankering on low- and high-spotting seedlings, the following experiment was performed. Six half-sib families known to exhibit a large variance in spotting incidence were grown and inoculated as 2-year-old seedlings in September 1990 following an established protocol (Meagher and Hunt 1993). Starting in January 1991, seedlings were examined weekly until 13 May for infection spots. To prevent cankering, the infected needles were removed as soon as spots appeared, however, many seedlings became cankered and needed pruning to eliminate them. Infection-spot tallies were finalized in November, and individual seedlings within a family were designated as either a high or a low spotter; high and low spotters from the same family were then planted in adjacent four-tree row plots (2 m apart) in a coastal site where *Ribes* spp. was present. Row plots of high and low spotters from different families alternated down the rows. Cankers on each tree were counted periodically with a final tally after 7 years. The cumulative mean canker values were calculated for the four trees in each plot and compared with the adjacent plot from the opposite spotting category of the same family.

### Experiment 2

*Artificially inoculated families*

Between 1986 and 1995, about 60 families were inoculated annually with a composite collection of coastal inoculum (Meagher and Hunt 1999). Family-spot means were derived from five half-sib seedling plots replicated 18 times. Infection success and overall spot means varied from year to year, and family means within years were generally skewed toward low values. Families were ranked by percentiles and these proportional values were transformed by the arcsine of the square root. Percentiles were determined by two different methods: (*i*) the overall infection spotting mean of all the seedlings within a family and (*ii*) the mean infection spotting from the average of each of the 18 plots. Percentile methods with transformation alleviated the skewed distribution and permitted family comparisons across years by Student – Newman – Keuls' (SNK) multiple-range test for a general linear model (GLM) (GLM–SNK procedure using SAS® (Cary, N.C.)) (Meagher and Hunt 1996). In 1995, four families already selected for re-

**Table 1.** Western white pine families, half-sibs per family, and rust (cankering) incidence in eight plantations, with correlation values between family cankering incidence in plantations and family spotting incidence from artificial inoculation with *Cronartium ribicola*.

| Plantation* | Family (no.) | Sibs/family (mean no.) | Rust (%) | Spearman's ($r_s$ value) |
|---|---|---|---|---|
| Okeover | 63 | 23 | 36 | 0.05 |
| Gowland | 18 | 21 | 57 | 0.64† |
| Theodosia | 15 | 60 | 22 | 0.23 |
| Texada A | 37 | 31 | 76 | –0.06 |
| Pender | 16 | 21 | 44 | –0.42 |
| Texada B | 16 | 25 | 46 | 0.38 |
| Smallwood L | 36 | 25 | 88 | –0.15 |
| Smallwood U | 22 | 58 | 41 | 0.38 |

*All plantations are coastal, except Smallwood L and U, which are near Nelson B.C. All are excess stock plantations, except Texada B.
†Significant at $P < 0.005$.

duced infection spotting by the Dorena Tree Improvement Centre were included in the study.

All surviving seedlings were held in pots in outdoor beds free from inoculum. They were observed annually or biennially for 3–5 years to determine family incidence of slow canker growth resistance (Hunt 1997). To maximize the slow canker-growth values per family, the few seedlings lacking cankers from the initial artificial inoculation were re-inoculated by placing them in a disease garden and observed for slow canker growth responses.

*Naturally inoculated families*

Starting with the 1992 annual inoculation, seedlings in excess of the needs for inoculation were established in "excess stock" plantations. Before 1992, 16 families from several different inoculation years were established in "demonstration" plantations. From 1997 to 1999, all trees in 15 such plantations were examined for blister rust cankers. Spearman's rank correlations were run (SAS®) between the incidence of cankers in each family in eight of these plantations and the incidence of both infection spotting and slow canker growth resistance in each family as determined by screening.

## Results

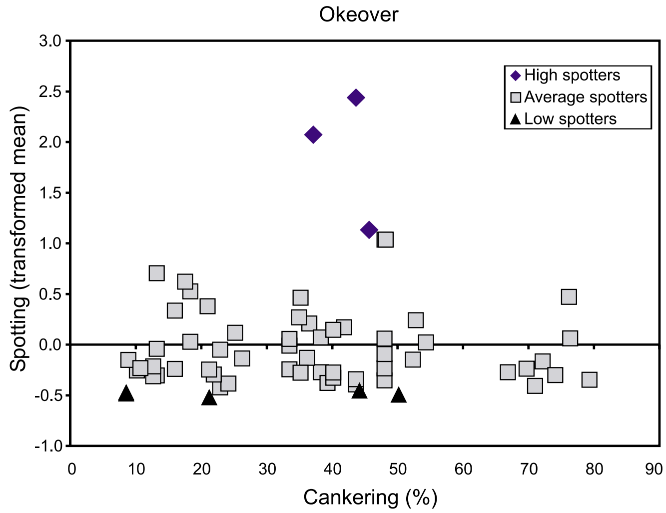
**Experiment 1**

The plantation contrasting paired high and low spotters had 26 of 29 pairs from six families with good survival. Of these, two had identical mean numbers of cankers, 12 of the high-spotting pairs had more cankers than the low-spotting pairs, and the other 12 high-spotting pairs had fewer cankers than their low-spotting counterparts. The overall mean number of cankers was 23 and 22 on low and high spotters, respectively.

**Experiment 2**

Seven of the excess stock plantations and one demonstration plantation had moderate to high incidence of blister rust cankering per family; the other plantations lacked sufficient rust for comparison and were excluded from the analysis. These eight plantations had blister rust incidence ranging from 22 to 88% (Table 1) and usually exhibited one canker per tree. Each plantation had 15 to 63 families with 16 or more half-sibs. Families with less than 16 half-sibs were excluded from the data. Although in one plantation there was a significant correlation between cankering incidence and spotting rank, most $r_s$ values were low and inconsistent with respect to sign and were non-significant (Table 1).

**Fig. 1.** Relationship between infection spotting of inoculated western white pine families and natural field cankering at the Okeover plantation (Spearman's $r_s = 0.05$; $P < 0.05$). The low- and high-spotting families were significantly different from all other families at $\alpha = 0.05$ by GLM–SNK analysis of transformed percentile values based on overall family spot means. Only the upper two high-spotted families were significantly separated from all other families by GLM–SNK analysis of transformed percentile values based on the means of 18 family plots at $\alpha = 0.05$.




The variance in most spotting data from inoculation was large, so few families were separated by the multiple range test. For families planted in the Okeover plantation (Fig. 1), three high spotters were significantly separated ($\alpha = 0.05$) from five low spotters using the overall family mean. The difference in field cankering between these two groups was not statistically different, being 37–45% for high spotters and 8–50% for low spotters. Using the plot mean values, the two most-spotted families were significantly separated from all others and their plantation cankering incidence was 37 and 43% compared with the overall range in cankering incidence of 8–79%. Similarly, the Texada A plantation had a pair of high-spotting families separated from a pair of low-spotting families using the overall seedling mean. These were 87 and 89% cankered verses 50 and 83% cankered, respectively. There were no significant differences using the plot means, and none of the other plantations had families with significant differences in spotting incidence based on inoculation testing.

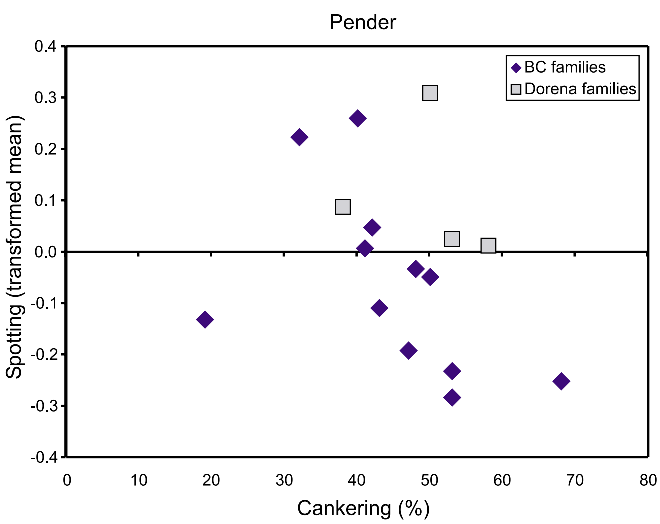
Four families that had already been selected for reduced infection spotting at the Dorena Tree Improvement Centre

**Table 2.** Correlation between slow canker growth (SCG) incidence in western white pine families as determined from artificial inoculation with *Cronartium ribicola* and field cankering in eight plantations, in conjunction with the incidence of field cankering in three family classes for slow canker growth resistance.

| Plantation | Spearman's ($r_s$ value)† | Mean cankering % per family* | | | Spearman's ($r_s$ value)‡ |
|---|---|---|---|---|---|
| | | 0% SGC | <5% SGC | >5% SGC | |
| Okeover | 0.26 (0.05) | 40.7 (22) | 35.9 (36) | 20.8 (5) | 1 |
| Gowland | 0.70 (0.001) | 73.8 (5) | 55.8 (9) | 39.8 (4) | 1 |
| Theodosia | 0.00 (1.00) | 20.2 (4) | 23.4 (10) | 11.8 (1) | 0.5 |
| Texada A | 0.27 (0.10) | 76.9 (21) | 79.7 (11) | 62.0 (5) | 0.5 |
| Pender | 0.53 (0.04) | 49.0 (13) | 47.0 (1) | 25.5 (2) | 1 |
| Texada B | 0.27 (0.31) | 48.6 (11) | 42.9 (3) | 36.0 (2) | 1 |
| Smallwood L | 0.23 (0.17) | 93.6 (9) | 88.1 (23) | 77.1 (5) | 1 |
| Smallwood U | 0.15 (0.51) | 41.4 (16) | 38.2 (4) | 38.0 (2) | 1 |
| Mean | | 55.5 (13) | 51.4 (12) | 38.9 (3) | 0.87 |

*Sample sizes (no. of families) appear in parentheses.
†*P*-values appear in parentheses.
‡Class ranks compared with expected ranks if cankering decreases with increasing SCG class. The mean is significant at $P < 0.01$.

**Fig. 2.** Relationship between infection spotting of inoculated western white pine families and natural field cankering at the Pender plantation (Spearman's $r_s$ = –0.42; $P < 0.10$). The USDA Forest Service had previously selected the Dorena families as low spotters.




had infection spotting percentiles from the artificial inoculation in B.C. ranging from 47 to 85, with a mean of 64. These were planted in one plantation and the mean cankering (50%) was not significantly different from the plantation mean (46%) (Fig. 2).

There was a weak trend in three of eight plantations for a significant positive correlation of less cankering in individual families with increasing slow canker growth resistance as determined from inoculations (Table 2). However, if the families were divided into slow canker growth classes, there was a trend of reduced cankering with increasing class of slow canker growth resistance (mean Spearman's $r_s$ value = 0.87 ($P < 0.05$) across all plantations). For the classes with less than 5% slow canker growth and greater than 5% slow canker growth the percentage reduction was 4 and 17, respectively (Table 2).

## Discussion

The mean infection spotting percentile for Dorena families (64) selected by the U.S. Forest Service for reduced spotting was above average (50) and the field cankering was slightly greater than the mean for unselected B.C. families. This suggests that the least-spotted families selected for reduced infection spotting (Meagher et al. 1990) in B.C. would be a more rigorous selection than at Dorena. However, in seven of eight plantations, there was no significant correlation between cankering and infection-spotting incidence after inoculation. Also, there was much overlapping among high- and low-spotting families for cankering incidence in plantations, even among families that were initially separated by a multiple-range test for infection spotting. The plantation established to contrast populations of high and low infection spotting was composed of the same families; thus, the genetic background should have been similar for each population except for infection-spotting ability. In this plantation there was no difference between these two populations for cankering incidence. These data indicate there is no practical gain in blister rust resistance by selecting families for reduced spotting. Moreover, the data do not support the notion (Hunt and Meagher 1993) of culling high-spotted families. There are several possibilities as to why reduced infection spotting does not result in less cankering in plantations. Western white pines close to inoculum sources are much more cankered than those further away (Hagle et al. 1989; Hunt 2000; Kimmey and Wagener 1961). The uneven distribution of inoculum in both the field and the inoculation chamber may result in a high variance that obscures the differences among families on a practical basis. The prime infection courts of older trees may be on older needles, whereas in the screening trials the infection courts are on current needles (Hunt and Jensen 2000). Additionally, infection success diminishes with increasing host age (Patton 1967). Thus, reduced infection spotting may only occur in juveniles (Hunt and Jensen 1999). It is also known that conifers tend to close their stomata during the winter (Smith et al. 1984), and because screening inoculations occur during one short infection episode in the fall, families might be differentially starting to close their stomata. Stomata are the infection courts for *C. ribicola* (Patton and Johnson 1970), so selecting families for reduced infection spotting maybe an indirect selection for early dormancy. In contrast, natural infection may be less affected by closing stomata, because it occurs over several episodes, particularly at the B.C. coast where infection may start in the spring (Hunt and Jensen 2000).

Selecting families for slow canker growth resistance was superior to selecting families for reduced infection spotting. It was surprising that there was a trend for fewer cankers in families in classes with greater slow canker growth resistance than in those families with no slow canker growth resistance, because the overall incidence of slow canker growth was very low (Table 2). This suggests that increasing the frequency of slow canker growth resistance within families, perhaps by crossing, may further decrease cankering incidence in plantations.

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