Differences in needle morphology between blister rust resistant and susceptible western white pine stocks

Kwan-Soo Woo, Lauren Fins, Geral I. McDonald, and Maurice V. Wiese

Abstract: Needle traits were evaluated on three groups of western white pine (*Pinus monticola* Dougl.) seedlings: four open-pollinated families that ranked high for the "reduced needle lesion frequency" type of resistance to blister rust; four blister rust susceptible families; and two bulk lots from a seed orchard selected for blister rust resistance. No statistically significant differences were found for most traits in pairwise comparisons among the three groups. However, needles of susceptible families had significantly wider and larger stomata (greater area) than did those of resistant families and seed orchard lots; their stomata were also rounder (smaller ratio of stomatal length to width) than those of the seed orchard lots. Needles of the resistant stocks were significantly shorter than those from seed orchard bulks. Contact angles of water droplets on adaxial needle surfaces were also significantly larger on resistant families compared with the other genetic stocks. Results suggest the possibility of some threshold stomatal size and (or) critical stomatal shape related to infection by the blister rust fungus, *Cronartium ribicola* J.C. Fisch. ex Rabenh., and possible differences among the groups in wax chemistry and (or) surface textures, both of which may alter behavior of blister rust germ tubes and (or) be altered by blister rust infection.

Résumé : Les caractères foliaires ont été évalués chez trois groupes de semis de pin blanc de l'Ouest (*Pinus monticola* Dougl.) : quatre familles à pollinisation libre classées élevées pour le type de résistance à la rouille vésiculeuse qui est caractérisée par une fréquence réduite des lésions sur les aiguilles, quatre familles sensibles à la rouille vésiculeuse et deux lots représentatifs sélectionnés dans un verger à graines pour leur résistance à la rouille vésiculeuse. Aucune différence statistiquement significative n'a été observée entre les trois groupes pour la plupart des caractères dans les comparaisons par paires. Cependant, les aiguilles des familles sensibles avaient des stomates significativement plus larges et plus grands (plus grande surface) que ceux des familles résistantes ou des lots provenant du verger à graines; leurs stomates étaient également plus ronds (plus petit ratio de la longueur sur la largeur des stomates) que ceux des lots provenant du verger à graines. Les aiguilles des semis résistants étaient significativement plus courtes que celles des lots provenant du verger à graines. L'angle de contact des gouttelettes d'eau sur la surface adaxiale des aiguilles était également significativement plus grand chez les familles résistantes comparativement aux autres groupes génétiques. Les résultats semblent indiquer qu'une certaine dimension seuil ou forme critique des stomates serait reliée à l'infection par le champignon, *Cronartium ribicola* J.C. Fisch. ex Rabenh., qui est responsable de la rouille vésiculeuse, et possiblement à des différences entre les groupes dans la composition chimique de la cire ou dans la texture de la surface qui peuvent toutes deux modifier le comportement du tube germinatif du pathogène ou être modifiées suite à l'infection par la rouille vésiculeuse.

[Traduit par la Rédaction]

Introduction

White pine blister rust, caused by the fungus *Cronartium ribicola* J.C. Fisch. ex Rabenh., was inadvertently introduced into western North America in 1910 on a single shipment of nursery stock of eastern white pine (*Pinus strobus* L.) imported from Ussy, France, to Point Grey, near Vancouver, B.C. (Mielke 1943). Western white pine (*Pinus monticola* Dougl.) proved highly susceptible to the disease, which spread rapidly through the white pine forests of southern British Columbia, eastern Washington, and northern Idaho.

Early attempts to control blister rust by eradication of *Ribes* species (the alternate hosts for the fungus) and the application of antibiotics largely failed (Maloy 1997). However, in the late 1940s, in the inland northwestern United States, a white pine selection and breeding program was initiated to enhance genetic resistance to blister rust (Bingham 1983). Seed orchard stocks produced by that program continue to outperform unimproved (susceptible) stocks in operational plantings and field tests throughout the region (Fins et al. 2001).

Received January 30, 2001. Accepted June 24, 2001. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on October 12, 2001.

K.-S. Woo and L. Fins.[1] Department of Forest Resources, University of Idaho, Moscow, ID 83844-1133, U.S.A.
G.I. McDonald. USDA Forest Service, Rocky Mountain Research Station, 1221 South Main Street, Moscow, ID 83843, U.S.A.
M.V. Wiese. Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844-2339, U.S.A.

[1]Corresponding author (e-mail: lfins@uidaho.edu).

 DOI: 10.1139/cjfr-31-11-1880 © 2001 NRC Canada

In a continuing effort to broaden the genetic base of resistance in the program, open-pollinated progeny of 200–250 candidate trees are tested and evaluated annually for resistance to blister rust (Mahalovich and Eramian 1995). At the end of the test, families are selected for horizontal resistance traits, defined as "partial resistance that is effective against most or all races (genetic variants) of a pathogen" (Shurtleff and Averre 1997). Horizontal resistance is likely to be under polygenic control and "durable" (Simmonds 1991; Messmer et al. 2000). Individuals within the families selected for horizontal resistance are further selected for vertical resistance traits, whose inheritance appears to be controlled by one or only a few genes (Simmonds 1991).

Forms of resistance that function by reducing rates of infection are known as "rate reducing resistance" (RRR) (Rossi et al. 1999). A type of RRR that reduces the number of needle spots that appear on white pine seedlings after exposure to blister rust is known to be under genetic control (Hoff and McDonald 1980; Meagher and Hunt 1996) and was shown to reduce infection efficiency by 10 times (Hoff and McDonald 1980). A long-term study of blister rust in a field planting, which showed the mean rate of infection in the F_2 improved stock to be six times lower than in the susceptible controls, provided direct evidence of RRR at work in the blister rust pathosystem (McDonald and Dekker-Robertson 1998).

Two of the traits for which families are selected in the Inland Empire program are based on numbers of needle spots that appear after artificial inoculation with *C. ribicola* basidiospores: "no spot" and "reduced needle lesion frequency" (RNLF). The no spot trait is evaluated as the proportion of seedlings with no needle spots (i.e., their needles remain uninfected) after the seedlings have been inoculated with blister rust spores; the RNLF trait is evaluated by the number of yellow needle spots that develop on the topmost fascicle after inoculation (expressed as "spots per metre" of needle length). However, the exact mechanisms by which seedlings avoid or reduce infection are not known.

Needle traits that block or slow the entry of *C. ribicola* have been suggested as factors that may reduce blister rust infection, but an early study comparing epidermal features of needles from four phenotypically clean (uninfected but untested) and four susceptible western white pine trees found no differences between them (Gansel 1956). A later study revealed that *C. ribicola* germ tubes enter needles through their stomata (Patton and Johnson 1970), suggesting that stomatal features are likely to be more important to the infection process than epidermal features. Whether there are differences in stomatal features of western white pine needles that are critical to infectability by blister rust is a question that, to date, remains unanswered.

One way needle morphology may affect the infection process is by influencing the amount of water that remains on its surface. "Wettability" refers to the tendency of a surface to retain water (nonwettable surfaces hold more water for longer periods of time than wettable surfaces). Differences in wettability may reflect differences pH, wax chemistry, and (or) surface structure (Leyton and Armitage 1968; Holloway 1969; Schreiber 1996). Since the amount and distribution of free moisture have been reported to be the most important factors for germination of *C. ribicola* basidiospores and subsequent infection of eastern white pine needles (Spaulding and Rathbun-Gravatt 1926; Hansen and Patton 1977), it seems reasonable that differences in wettability could also be indicators of the propensity of western white pine needles to become infected. Because contact angles of water droplets are higher on nonwettable surfaces than on wettable ones, contact angle can be used as a measure of surface "wettability" (Cape 1983; Leyton and Juniper 1963).

A routine nursery-based test of blister rust resistance in western white pine provided an ideal opportunity to test the hypotheses of critical differences in stomatal and other needle traits and surface wettability between blister rust susceptible stocks and stocks with needle-based resistance traits.

Materials and methods

Morphological characteristics

Needle samples were collected from western white pine seedlings (4.5 years from seed) grown at the USDA Forest Service nursery in Coeur d'Alene, Idaho, as part of a routine rust screening operation. Seedlings had been grown in 164-cm^3 single Super Cells™ in a greenhouse for two growing seasons, were inoculated with blister rust in August 1994 and transplanted to five outdoor nursery beds in May 1995.

A total of 271 entries (open-pollinated families, woods-run check lots, and open-pollinated bulk seed orchard lots) were included in the nursery planting. Entries were planted in contiguous plots, which were randomized within each of three replications (blocks), with a maximum of 35 seedlings per entry per plot. Seedlings were watered, weeded, and fertilized during the screening process.

Entries were ranked for the needle lesion frequency resistance trait based on the spring 1995 count of the number of yellow spots (infection sites) observed on one terminal fascicle from each of 15 randomly selected seedlings per replication (total 45 seedlings) (Mahalovich and Eramian 1995). Using these rankings, four resistant and four susceptible families and two seed orchard lots were selected for this study. The resistant group included four families that ranked high for RNLF resistance (families 3062, 3653, 4437, and 4922), one of which (4437) also ranked high for the no spot trait (Table 1). The susceptible group (families 3162, 3233, 4110, and 4778) all had a high incidence of needle lesions. The two F_2 open-pollinated bulk seed orchard lots came from an orchard in which the trees had been selected primarily for resistance traits other than RNLF resistance (Bingham 1983). Lot 4815 was a general seed orchard collection, but lot 4816 was collected from parent trees that had been selected for the "short shoot" type of resistance (needle lesions appear after inoculation but rust infection is stopped between the needle and the branch) (Rust 1998). Both seed orchard collections ranked ≥90th percentile for horizontal blister rust resistance in two rust screenings completed in 1996 and 1997 (Rust 1998).

Needle samples were collected from all 10 genetic stocks on October 16 and 17, 1997, and were stored for up to 2 weeks in plastic bags at 4°C until they were examined. We collected 3 current fascicles per seedling from each of 21 seedlings per source (7 seedlings per source per replication). One randomly chosen needle from each of the three fascicles per seedling was used for each part of this study, except where noted.

Stomatal characteristics were assessed using two 1-mm sample plots at a random distance from the center of each of the two adaxial surfaces (total of four 1-mm plots per needle). Needle characteristics were evaluated using a stereomicroscope at 62.5× magnification. Needle length (NL) was measured with a ruler to the

© 2001 NRC Canada

nearest 1.0 mm. A calibrated grid (eyepiece) was used to measure the width of two central adaxial sides (NW1 and NW2) to the nearest 0.05 mm and to count the number of stomata per millimetre and number of stomatal rows. Needle length and width measurements were used to estimate adaxial surface area (AdSA). In each plot, the number of stomata per row in two randomly selected rows was counted and the average number of stomata per row (ANS) was calculated. Total number of stomata per needle (ETNS) was estimated assuming the needle had a constant number of stomatal rows and a rectangular shape. Some needles had a few stomata on the abaxial side (outer needle surface), but only the adaxial stomatal traits (ETNS and AdSA) were used to determine stomatal density (SD = ETNS/AdSA).

Stomatal length (major axis, μm) and width (minor axis, μm) were measured directly through the monitor of a scanning electron microscope (SEM) (Hitachi S-570 at 10–15 kV magnification at 500× and 15 mm working distance). The center portion of each needle was prepared for SEM by cryofixation and lyophilized at –55°C for 12 h (Larsen and Green 1992). Dried needle specimens were coated with gold to approximately 1–2 nm thick (10–20 Å) in a Polaron sputter coater. Two stomatal rows were randomly selected on each adaxial side of the needle, and five randomly chosen stomata were measured per row to the nearest 1 μm. Stomatal shape, generally elliptical, was described by the ratio of the length of the major axis divided by the length of the minor axis. Stomatal areas were calculated using the standard formula for the area of an ellipse (Mitton et al. 1998) as

$$[1]\quad \text{Stomatal area}\,(\mu m^2) = 3.14 \times \left(\frac{\text{major axis}\,(\mu m)}{2}\right) \times \left(\frac{\text{minor axis}\,(\mu m)}{2}\right)$$

Assessments of differences in stomatal occlusion (blockage of the stomatal opening by wax) and stomatal size, shape, and area were based on observations of 10 stomata on one needle per seedling on each of 21 seedlings per seed source (five stomata per row on two randomly selected rows on one current-year needle per seedling, seven seedlings per replication, three replications).

The structure and distribution of surface waxes around the stomata were observed with a stereomicroscope (62.5×) and SEM (120×). Stomata were classified as open, partially open, or occluded. Epistomatal waxes were classified by percentage of fine versus fused (degraded) wax crystallites using a five-category classification method (Trimbacher and Eckmüllner 1997).

Needle wettability

The wettability of current-year needles was assessed as a function of the contact angles of water droplets placed on the two adaxial surfaces (inner, flat sides of each needle). Only the adaxial sides were used, because stomata were infrequent on the abaxial surfaces (Woo 2000).

Contact angles were measured on four droplets of distilled water per needle (two on each of the two adaxial surfaces). Needles were brought to room temperature, and their surfaces were allowed to dry prior to application of water droplets (2 μL each at 22°C), which were applied with a micropipette (or a microlitre syringe) and observed under 50× magnification. Water droplets were placed on other parts of the needle surface if they did not adhere to the center part. Contact angles were measured with a protractor at 75× on a television monitor connected to a video camera and a dissecting microscope. The left and right angles were averaged for each water droplet.

Statistical analyses

Data were analyzed using PROC GLM (general linear model) in the SAS statistical package (SAS Institute Inc. 1989). The exploratory PROC UNIVARIATE test was done on all residuals (r_{ijk} = $y_{ijk} - \hat{y}_{ijk}$) to test the assumption of a normal distribution. A randomized block hierarchical design with seedlings nested within the block × family interaction was used to test differences among genetic stocks (Kirk 1995). The analytical model for the analysis was

$$[2]\quad Y_{ijkl} = \mu + \alpha_i + \pi_j + (\alpha\pi)_{ij} + \delta_{k(ij)} + \varepsilon_{l(ijk)}$$

where, Y_{ijkl} is the observation of the lth needle of the kth seedling in the jth block and ith family, μ is the overall sample mean, α_i is the effect of ith family, π_j is the effect of jth block, $(\alpha\pi)_{ij}$ is the interaction of the jth block and ith family, $\delta_{k(ij)}$ is the effect of the kth seedling nested in the block × family interaction, and $\varepsilon_{l(ijk)}$ is the residual representing variability within seedlings.

For the proportion of occluded stomata, data were transformed by arcsine as

$$[3]\quad Y = \arcsin(P)^{0.5}$$

where P is the proportion of occluded stomata per seedling and Y is the new variable (SAS Institute Inc. 1989; Ott 1993). Values for the contact angles were the average of four measurements per side (left and right angles of two water droplets) for the two adaxial surfaces of each needle.

When the GLM test showed significant differences among the 10 genetic stocks at $p \le 0.10$, the three groups of genetic stocks (resistant, susceptible, and bulked lots) were contrasted in nonorthogonal (dependent) pairwise comparisons. The p values shown in Table 3 were adjusted by the Bonferroni method of multiplying the raw p values by the number of tests conducted.

Table 1. Rust infection frequency in western white pine seedlings from resistant and susceptible families and seed orchard F_2 bulk lots.

Families and bulked lots	Spot frequency[a]	No. of seedlings[b]	No. with zero spots[c]	Zero spots (%)
Resistant				
3062	0.00	103	46	45
3653	0.00	105	19	18
4437	0.26	81	59	73
4922	0.00	105	24	23
Susceptible				
3162	4.10	104	37	36
3233	3.51	105	32	30
4110	2.79	105	35	33
4778	2.88	84	32	38
Bulk lots[d]				
4815[e]	0.00	103	76	74
4816	0.40	103	36	35

[a]Spot frequency at first spring inspection after inoculation = (yellow spot frequency)/(length of fascicle (mm) from top of seedling × 5) × 1000.
[b]Total number of seedlings in test.
[c]Number of seedlings with no spots 1 year after inoculation.
[d]From R.T. Bingham White Pine Seed Orchard in Moscow, Idaho.
[e]Reported as 66% resistant to blister rust at 2.5 years after inoculation (Hoff et al. 1973).

Results

Morphological characteristics

The 10 seed sources differed significantly from each other ($p \le 0.10$) for nearly all of the traits evaluated in this study (Table 2). Pairwise comparisons between groups of genetic stocks (resistant vs. susceptible vs. seed orchard bulks) showed stomata of the susceptible group to be significantly wider and greater in area than those of the resistant group

© 2001 NRC Canada

Table 2. Means and SEs (in parentheses) of needle traits of western white pine seedlings from two seed orchard F_2 bulk lots, and four blister rust resistant and four blister rust susceptible western white pine families.

	F_2 seed orchard bulk lots		Resistant families				Susceptible families				
Trait[a]	4815	4816	3062	3653	4437	4922	3162	3233	4110	4778	p[b]
NL (mm)	77.32	73.98	65.38	73.14	70.94	73.02	74.83	69.78	74.21	77.26	0.0096
	(1.69)	(1.17)	(1.22)	(1.13)	(0.99)	(1.40)	(1.64)	(1.60)	(1.38)	(1.71)	
NW1 (mm)	0.81	0.79	0.78	0.81	0.79	0.85	0.81	0.83	0.79	0.77	0.0047
	(0.013)	(0.008)	(0.008)	(0.009)	(0.008)	(0.010)	(0.011)	(0.013)	(0.013)	(0.013)	
NW2 (mm)	0.80	0.80	0.77	0.81	0.79	0.85	0.81	0.82	0.79	0.78	0.0284
	(0.014)	(0.009)	(0.008)	(0.009)	(0.008)	(0.010)	(0.011)	(0.013)	(0.013)	(0.013)	
MNR	3.62	3.59	3.39	3.74	3.45	3.80	3.52	3.73	3.58	3.50	0.0202
	(0.058)	(0.059)	(0.05)	(0.066)	(0.052)	(0.076)	(0.06)	(0.066)	(0.071)	(0.055)	
MNS	14.45	14.35	14.75	14.77	14.66	14.83	13.97	15.0	14.82	15.21	0.0001
	(0.142)	(0.141)	(0.131)	(0.131)	(0.131)	(0.155)	(0.135)	(0.142)	(0.147)	(0.16)	
ETNS	8091.6	7615.1	6517.2	8125.6	7172.7	8286.2	7430.7	7929.4	7911.4	8312.6	0.0161
	(243.0)	(184.3)	(149.3)	(235.8)	(157.4)	(272.8)	(279.5)	(288.6)	(267.5)	(291.7)	
AdSA (mm^2)	126.73	118.11	101.45	118.97	112.11	125.03	122.39	116.65	118.68	121.74	0.0704
	(4.46)	(2.47)	(2.41)	(2.60)	(2.10)	(3.46)	(4.18)	(3.89)	(3.96)	(4.31)	
SD	65.29	64.59	64.87	68.25	64.12	66.29	60.81	68.26	67.14	69.11	0.0028
	(1.26)	(0.94)	(1.04)	(1.23)	(0.93)	(1.23)	(0.95)	(1.23)	(1.02)	(1.30)	
Major axes (μm)	58.24	59.26	59.42	57.54	59.00	58.07	60.68	59.30	56.97	58.64	0.0181
	(0.50)	(0.59)	(0.68)	(0.79)	(0.92)	(0.84)	(0.53)	(0.64)	(0.72)	(0.87)	
Minor axes (μm)	34.45	34.45	35.42	35.80	34.97	34.70	36.10	36.00	35.25	37.94	0.0001
	(0.42)	(0.40)	(0.47)	(0.53)	(0.65)	(0.36)	(0.42)	(0.40)	(0.37)	(0.51)	
Major/minor axes	1.70	1.73	1.70	1.62	1.71	1.68	1.69	1.66	1.63	1.56	0.0008
	(0.03)	(0.03)	(0.03)	(0.03)	(0.05)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	
Stomatal area (μm^2)	1574.54	1603.10	1650.2	1619.43	1618.54	1582.29	1718.06	1674.29	1576.53	1746.51	0.0001
	(22.68)	(24.42)	(24.01)	(38.70)	(36.38)	(27.79)	(22.90)	(26.47)	(25.10)	(32.87)	
Stomatal occlusion	95.7	96.2	93.8	95.7	94.8	93.8	98.6	96.7	93.3	91.9	0.38
	(0.02)	(0.01)	(0.03)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.02)	(0.02)	
Degraded wax	2.30	2.62	2.58	3.11	2.62	3.05	2.79	2.73	2.83	2.92	0.27
	(0.17)	(0.19)	(0.26)	(0.24)	(0.16)	(0.23)	(0.24)	(0.27)	(0.24)	(0.20)	
Contact angle (°)	99	99	107	99	104	101	101	98	101	94	0.0001
	(0.60)	(0.71)	(0.65)	(0.78)	(0.49)	(0.88)	(0.73)	(1.07)	(0.48)	(1.03)	

Note: All genetic stocks were open pollinated. Resistant group included four families that ranked high for "reduced needle lesion frequency" resistance (one of which also ranked high for the "no spot" resistance trait). The susceptible group included four families with high infection rates from the blister rust fungus. Seed orchard lots were from two collections of F_2 seed from the R.T. Bingham Western White Pine Seed Orchard in Moscow, Idaho. One of the seed orchard lots was a general collection; the other was from trees selected for the short shoot resistance mechanism.

[a]NL, needle length; NW1, needle width 1 on adaxial side; NW2, needle width 2 on adaxial side; MNR, mean number of stomatal rows per plot; MNS, mean number of stomata per row (two rows selected randomly in each plots); ETNS, estimated total number of stomata per needle (MNR × MNS × NL × 2); AdSA, adaxial surface area (NL × (NW1 + NW2)); SD, stomatal density (ETNS/AdSA); major axes, long axes of stomata; minor axes, short axes of stomata; major/minor axes, stomatal shape index; stomatal area, calculated as 3.14 × (major axis/2) × (minor axis/2); stomatal occlusion, percentage of stomata occluded by wax; degraded wax, mean score on a five-point scale from <10% degradation (1) to >75% degradation (5) of waxes around stomata; contact angles, degree of the angle of contact of a water droplet with the needle surface on which it rests.

[b]The p values refer to comparisons among all 10 genetic stocks.

and seed orchard lots (Table 3). They were also "rounder" (smallest ratio of major to minor axes) than stomata on the seed orchard bulk lots (Table 3). Stomata on needles from the seed orchard lots were relatively narrow and had the smallest stomatal area. Needles from the resistant families were significantly shorter than those from seed orchard bulk lots (Table 3).

Wax characteristics

Epicuticular wax crystallites appeared to be tubular with hollow centres or were fused and platelike (Woo 2000). Most of the crystallites ranged in diameter from 0.09 to 0.18 μm. Generally, more wax was visible on the distal third of the adaxial surfaces than in the middle or proximal ends. Larger wax deposits were common along stomatal rows and over epistomatal chambers (where they were degraded primarily in the peripheral areas, with wax crystallites intact beneath the encrustations).

Fine-structured or fused wax occluded more than 90% of stomata on needles from all 10 genetic stocks, with no significant differences among sources in occlusion or in the proportions of degraded epistomatal wax (Table 2).

Needle wettability

Mean contact angles of water droplets were statistically different among the 10 genetic stocks (Table 2), ranging from 107° (family 3062) to 94° (family 4778), and were significantly larger on resistant families than on susceptible

© 2001 NRC Canada

Table 3. Means, SEs (in parentheses), and pairwise comparisons of eight needle characteristics between four blister rust resistant (R) and four blister rust susceptible (S) western white pine families and two F_2 seed orchard bulk lots (M).

Trait[a]	Means and SEs			Pairwise comparison	p[b]
	R	S	M		
NL (mm)	70.619	74.004	75.651	R vs. S	0.107
	(0.626)	(0.808)	(1.037)	R vs. M	0.015
				S vs. M	1
NW1 (mm)	0.807	0.799	0.803	R vs. S	1
	(0.005)	(0.006)	(0.007)	R vs. M	1
				S vs. M	1
NW2 (mm)	0.803	0.80	0.799	R vs. S	1
	(0.005)	(0.006)	(0.008)	R vs. M	1
				S vs. M	1
MNR	3.595	3.583	3.605	R vs. S	1
	(0.033)	(0.032)	(0.042)	R vs. M	1
				S vs. M	1
MNS	14.754	14.746	14.401	R vs. S	1
	(0.068)	(0.079)	(0.10)	R vs. M	0.068
				S vs. M	0.084
ETNS	7525.42	7894.35	7853.38	R vs. S	0.508
	(114.03)	(141.79)	(153.37)	R vs. M	0.678
				S vs. M	1
AdSA (mm^2)	114.392	119.854	122.420	R vs. S	1
	(1.45)	(2.04)	(2.57)	R vs. M	0.125
				S vs. M	1
SD	65.883	66.318	64.943	R vs. S	1
	(0.56)	(0.60)	(0.78)	R vs. M	1
				S vs. M	0.778
Major axes (μm)	58.5	58.9	58.8	R vs. S	1
	(0.4)	(0.4)	(0.4)	R vs. M	1
				S vs. M	1
Minor axes (μm)	35.2	36.3	34.5	R vs. S	0.002
	(0.3)	(0.2)	(0.3)	R vs. M	0.152
				S vs. M	0.0003
Major/minor axes	1.68	1.64	1.72	R vs. S	0.126
	(0.017)	(0.015)	(0.019)	R vs. M	0.314
				S vs. M	0.002
Stomatal area (μm^2)	1617.6	1678.9	1588.8	R vs. S	0.012
	(16.1)	(15.1)	(16.6)	R vs. M	0.779
				S vs. M	0.0003
Contact angle (°)	102.6	98.6	99.2	R vs. S	0.0003
	(0.4)	(0.5)	(0.5)	R vs. M	0.0003
				S vs. M	1

Note: All genetic stocks are open pollinated. Resistant group (R) included four families that ranked high for "reduced needle lesion frequency" resistance (one of which also ranked high for the "no spot" resistance trait). Susceptible group (S) included four families with high infection rates from the blister rust fungus. Seed orchard lots (M) were from two collections of F_2 seed from the R.T. Bingham Western White Pine Seed Orchard in Moscow, Idaho. One of the seed orchard lots was a general collection; the other was from trees selected for the short shoot resistance mechanism.

[a]See Table 2 for definitions of variables.

[b]The p values shown were adjusted by the Bonferroni method of multiplying the raw p values by the number of tests conducted.

families and seed orchard bulk lots, which were not statistically different from each other (Table 3).

Discussion

Although the suite of traits we evaluated was somewhat different from those studied by Gansel (1956), our findings are generally consistent with his, i.e., there is little difference in needle traits between susceptible and resistant genetic stocks. However, our finding that susceptible families have larger and rounder stomata than resistant stocks is noteworthy. If small or narrow stomata act as barriers to entry of blister rust basidiospore germ tubes, stomatal size, particularly at some threshold, may be an important and po-

© 2001 NRC Canada

tentially definitive phenotypic trait that could be used to identify RNLF resistance genotypes.

Rust resistance in some plants, such as wheat, can also be related to timing, duration, and completeness of stomatal opening and closure (Hart 1929). Stomata that open slowly or remain open for only short periods of time can limit opportunities for germ tubes to enter the needle, whereas stomata that close slowly or incompletely may be penetrated more easily. Observations of stomatal behavior were outside the scope of our study, and no such relationships were found between blister rust infection and eastern white pine (Hirt 1938); however, it is possible that the differences we found in stomatal size and shape have behavioral correlates that are important to the infection process. The potential for such a relationship warrants further investigation.

Consistent with previous findings for other conifers, including eastern white pine (Hanover and Reicosky 1971; Trimble et al. 1982), we observed structural (threadlike or fibrillar) and platelike (amorphous) waxes on needles from western white pine seedlings. At high magnifications, some of the threadlike crystallites appeared tubular with hollow centers. This structure has previously been observed on needles of other conifers (Leyton and Juniper 1963; Leyton and Armitage 1968; Günthardt-Goerg et al. 1994).

Larger amounts of wax were deposited on the distal third of the adaxial sides of the needles compared with the central and proximal parts. Our observation of degraded waxes primarily near the periphery of epistomatal chambers, with intact crystallites beneath, is consistent with those of Crossley and Fowler (1986) on *Pinus sylvestris* L. and suggests that wax degradation begins on the periphery of the stomata and progresses inward.

Stomatal occlusion by wax has been suggested as a mechanism that may reduce the chance of blister rust infection in eastern white pine (Patton and Johnson 1970; Patton and Spear 1980), but with more than 90% of the stomatal chambers occluded and no differences among the 10 genetic stocks in either the proportions of stomatal occlusion or the proportions of degraded epistomatal wax, this seems an unlikely explanation for differences in infection between groups.

While the difference in contact angles of water droplets might be expected in a comparison between the resistant families and the susceptible families, the significant difference between the resistant families and the seed orchard bulk lots was not anticipated. This result may reflect a broader array of resistance mechanisms in the seed orchard bulks compared with the resistant families, especially since one of the seed orchard bulk lots was collected from parent trees that had been selected specifically for the short shoot resistance mechanism. If the structure of needle surfaces on genotypes with needle-based resistance mechanisms is different from those with mechanisms that operate later in the process, we would expect to find (as we did) differences in wettability between the seed orchard lots and the resistant families.

The genetic stocks included in this study came from a routine nursery rust screening that was sampled in the fifth and final year of the test. This timing allowed us to identify susceptible families and families with needle-based resistance mechanisms, all of which had been exposed to the same environmental conditions. Nonetheless, the needles sampled, i.e., current-year needles, were not the ones that had been exposed to blister rust during the inoculation process. There is some evidence that spotting frequency is affected by age of the needles (Hunt 1991). Physical structure of waxes and their chemical composition may change as the tree ages or is exposed to environmental pollutants (Percy and Riding 1978; Trimble et al. 1982; Riding and Percy 1985). Blister rust infection can itself alter the chemical composition of needle tissues (Martin 1972).

The differences we observed between genetic stocks are intriguing and potentially important. They suggest but do not prove critical relationships between infectability by blister rust, stomatal size and (or) shape, and surface wettability. If these traits are indeed relevant to the infection process, a determination of critical or threshold values for stomatal size and contact angles of surface water droplets will be important to our understanding of the disease process and for using the traits as screening criteria in selection and breeding programs.

Our research included only a small number of families in each of the three categories of resistance. We do not know whether the differences we observed are consistent across a larger array of families, or whether they are stable over time and (or) across a variety of environmental conditions. If our findings indeed prove relevant to the disease process, and if they are stable and diagnostic for resistance, it may be possible to reduce screening time for the reduced needle lesion frequency resistance trait by at least 1 year.

Acknowledgements

This study was supported by the Inland Empire Tree Improvement Cooperative based in Moscow, Idaho, U.S.A. The authors also warmly acknowledge Richard A. Sniezko for his much-appreciated comments on an earlier version of the manuscript.

References

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: the story of the first 25 years of the research and development program. USDA For. Serv. Gen. Tech. Rep. INT-146.

Cape, J.N. 1983. Contact angles of water droplets on needles of Scots pine (*Pinus sylvestris*) growing in polluted atmospheres. New Phytol. **93**: 293–299.

Crossley, A., and Fowler, D. 1986. The weathering of Scots pine epicuticular wax in polluted and clean air. New Phytol. **103**: 207–218.

Fins, L., Byler, J., Ferguson, D., Harvey, A., Mahalovich, M.F., McDonald, G., Miller, D., Schwandt, J., and Zack, A. 2001. Return of the giants. College of Natural Resources, University of Idaho, Moscow. Idaho For. Wildl. Range Exp. Stn. Bull. 72.

Gansel, C.R. 1956. A comparison of some morphological features of needles from blister rust- resistant and non-resistant western white pine. M.Sc. Thesis, University of Idaho.

Günthardt-Goerg, M.S., Keller, T., Matyssek, R., and Scheidegger, C. 1994. Environmental effects on Norway spruce needle wax. Eur. J. For. Pathol. **24**: 92–111.

Hanover, J.W., and Reicosky, D.A. 1971. Surface wax deposits on foliage of *Picea pungens* and other conifers. Am. J. Bot. **58**: 681–687.

© 2001 NRC Canada

Hansen, E.M., and Patton, R.F. 1977. Factors important in artificial inoculation of *Pinus strobus* with *Cronartium ribicola*. Phytopathology, **67**: 1108–1112.

Hart, H. 1929. Relation of stomatal behavior to stem-rust resistance in wheat. J. Agric. Res. **39**: 929–948.

Hirt, R.R. 1938. Relation of stomata to infection of *Pinus strobus* by *Cronartium ribicola*. Phytopathology, **28**: 180–190.

Hoff, R.J., and McDonald, G.I. 1980. Resistance to *Cronartium ribicola* in *Pinus monticola*: reduced needle-spot frequency. Can. J. Bot. **58**: 574–577.

Hoff, R.J., McDonald, G.I., and Bingham, R.T. 1973. Resistance to *Cronartium ribicola* in *Pinus monticola*: structure and gain of resistance in the second generation. USDA For. Serv. Res. Note INT-178.

Holloway, P.J. 1969. Chemistry of leaf waxes in relation to wetting. J. Sci. Food Agric. **20**: 124–128.

Hunt, R.S. 1991. The effect of age on the susceptibility to blister rust of western white pine seedlings. *In* Rusts of Pine. Proceedings of the 3rd IUFRO Rusts of Pine Working Party Conference, 18–22 Sept. 1989, Banff, Alta. *Edited by* Y. Hiratsuka, J.K. Samoil, P.V. Blenis, P.E. Crane, and B.L. Laishley. For. Can. North. For. Cent. Inf. Rep. NOR-X-317. pp. 287–290.

Kirk, R.E. 1995. Experimental design: procedures for the behavioral sciences. 3rd ed. Brooks/Cole Publishing Co., Pacific Grove, Calif.

Larsen, M.J., and Green, F., III. 1992. Microfibrillar cell wall extensions in the hyphal sheath of *Postia placenta*. Can. J. Microbiol. **38**: 905–911.

Leyton, L., and Armitage, I.P. 1968. Cuticle structure and water relations of the needles of *Pinus radiata* (D. Don). New Phytol. **67**: 31–38.

Leyton, L., and Juniper, B.E. 1963. Cuticle structure and water relations of pine needles. Nature (London), **198**: 770–771.

Mahalovich, M.F., and Eramian, A. 1995. Breeding and seed orchard plan for the development of blister rust resistant white pine for the northern Rockies. Draft tree improvement plan. USDA Forest Service Northern Region, Missoula, Mont.

Maloy, O.C. 1997. White pine blister rust control in North America: a case history. Annu. Rev. Phytopathol. **35**: 87–109.

Martin, N.E. 1972. Influence of blister rust on inorganic solute concentrations in western white pine. Phytopathology, **62**: 226–229.

McDonald, G.I., and Dekker-Robertson, D.L. 1998. Long-term differential expression of blister rust resistance in western white pine. *In* Proceedings of the 1st IUFRO Rusts of Forest Trees WP Conference, 2–7 Aug. 1998, Saariselka, Finland. Finnish Forest Research Institute, Rovaniemi. Res. Pap. 712. pp. 285–295.

Meagher, M.D., and Hunt, R.S. 1996. Heritability and gain of reduced spotting vs. blister rust on western white pine in British Columbia, Canada. Silvae Genet. **45**: 75–81.

Messmer, M.M., Seyfarth, R., Keller, M., Schachermayr, G., Winzeler, M., Zanetti, S., Feuillet, C., and Keller, B. 2000. Genetic analysis of durable leaf rust resistance in winter wheat. Theor. Appl. Genet. **100**: 419–431.

Mielke, J.L. 1943. White pine blister rust in western North America. Yale Univ. Sch. For. Bull. 52.

Mitton, J.B., Grant, M.C., and Yoshino, A.M. 1998. Variation in allozymes and stomatal size in pinyon (*Pinus edulis*, Pinaceae), associated with soil moisture. Am. J. Bot. **85**: 1262–1265.

Ott, R.L. 1993. An introduction to statistical methods and data analysis. 4th ed. Duxbury Press, Wadsworth Publishing Co., Belmont, Calif.

Patton, R.F., and Johnson, D.W. 1970. Mode of penetration of needles of eastern white pine by *Cronartium ribicola*. Phytopathology, **60**: 977–982.

Patton, R.F., and Spear, R.N. 1980. Stomatal influences on white pine blister rust infection. Phytopathol. Mediterr. **19**: 1–7.

Percy, K.E., and Riding, R.T. 1978. The epicuticular waxes of *Pinus strobus* subjected to air pollutants. Can. J. For. Res. **8**: 474–477.

Riding, R.T., and Percy, K.E. 1985. Effects of SO_2 and other air pollutants on the morphology of epicuticular waxes on needles of *Pinus strobus* and *Pinus banksiana*. New Phytol. **99**: 555–563.

Rossi, V., Giosue, S., and Racca, P. 1999. A model integrating components of rate-reducing resistance to Cercospora leaf spot in sugar beet. J. Phytopathol. **147**: 339–346.

Rust, M. 1998. Performance of Moscow seed orchard lots in artificial inoculation trials. *In* the Inland Empire Tree Improvement Cooperative 22nd progress report. *Edited by* L. Fins and M. Rust. University of Idaho, Moscow, Idaho.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6. 4th ed. SAS Institute Inc., Cary, N.C.

Schreiber, L. 1996. Wetting of the upper needle surface of *Abies grandis*: influence of pH, wax chemistry and epiphyllic microflora on contact angles. Plant Cell Environ. **19**: 455–463.

Shurtleff, M.C., and Averre, C.W., III. 1997. Glossary of plant-pathological terms. American Phytopathological Society, St. Paul, Minn.

Simmonds, N.W. 1991. Genetics of horizontal resistance to diseases of crops. Biol. Rev. **66**: 198–241.

Spaulding, P.C., and Rathbun-Gravatt, A. 1926. The influence of physical factors on the viability of sporidia of *Cronartium ribicola* Fischer. J. Agric. Res. **33**: 397–433.

Trimbacher, C., and Eckmüllner, O. 1997. A method for quantifying changes in the epicuticular wax structure of Norway spruce needles. Eur. J. For. Pathol. **27**: 83–93.

Trimble, J.L., Kelly, J.M., Tolin, S.A., and Orcutt, D.M. 1982. Chemical and structural characterization of the needle epicuticular wax of two clones of *Pinus strobus* differing in sensitivity to ozone. Phytopathology, **12**: 652–656.

Woo, K.S. 2000. Variation in needle morphology of blister rust susceptible and resistant western white pine. Ph.D. dissertation, University of Idaho, Moscow.

© 2001 NRC Canada