Rust infection and survival of 49 *Pinus monticola* families at a field site six years after planting

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Kolpak S.E., Sniezko R.A., Kegley A.J., 2008. Rust infection and survival of 49 *Pinus monticola* families at a field site six years after planting. Ann. For. Res. 51: 67-80.

Abstract. This field trial evaluates white pine blister rust resistance of 49 families of Pinus monticola from Oregon and Washington. The Optical site had 93% of the trees infected six years after planting, but rust mortality is currently low. Families ranged between 31 to 100 percent infected and the number of stem symptoms ranged between three and eighteen. Families with *R-gene* resistance (HR) from the Cr2 gene were highly susceptible at Optical due to the presence of a virulent race of rust (vcr2). Families without Cr2 and with moderate to high frequencies of canker-free or bark reaction ('Q' families) in seedling screening at Dorena Genetic Resource Center (DGRC) were the most resistant at Optical with higher bark reaction and lower percent infection, mortality, and number of stem symptoms. Another subset of the families were inoculated in a seedling trial at DGRC using rust strains not containing *vcr2*. The *Cr2* families had predictable levels of resistance and Q families also showed moderate levels of resistance in the DGRC seedling trial. Percent stem symptom and bark reaction traits were positively correlated between screening and field trials. Also the number of needle lesions in artificial screening was positively correlated with the number of stem symptoms at Optical.

Keywords: *Pinus monticola, Cronartium ribicola*, white pine blister rust, *R-gene* resistance, bark reaction, field trials

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Introduction

Western white pine (*Pinus monticola* Dougl.) historically made up a larger component of forests in western North America. Several interacting factors have contributed to its decline including logging, white pine blister rust (caused by the non-native, invasive pathogen *Cronartium ribicola* J.D. Fisch. in Rabenh.), fire exclusion, and mountain pine beetle (*Dendroctonus ponderosae* Hopk.) (Bower & Sniezko 2004, McDonald et al. 2004, Fins et al. 2002). White pine blister rust (WPBR) is a major obstacle for the restoration

of western white pine (WWP) due to the high susceptibility of this and other five-needle pine species (Hoff et al. 1980, Kegley & Sniezko 2004). Descriptions of the introduction and the spread of the WPBR have been reviewed (McDonald & Hoff 2001, Kinloch et al. 2003). The development of populations of WWP genetically resistant to WPBR is viewed as key to initiating restoration and use in reforestation. Several well established operational resistance programs in western North America are developing genetically resistant five-needle pines (King & Hunt 2004, Sniezko 1996, McDonald et al. 2004, Fins et al. 2002,

Samman & Kitzmiller 1996).

Long-term monitoring of resistant trees on WPBR infected field sites is a critical component of operational breeding programs. Field plantings can serve a variety of purposes (c.f. Sniezko 1996) including: monitoring changes in virulence of WPBR, planning deployment strategies, identifying ontogenetic resistance, assessing the durability of resistance, and validating rust responses of families characterized in short-term tests. The latter is important because screening trials often use a single inoculation with rust on young seedlings, fairly uniform conditions, high levels of inoculum, and a mixture of rust sources. In contrast, infections in field trials can occur over a period of years, on older trees, over a range of environmental conditions, and with different mixtures of rust sources (Sniezko et al. 2004a).

WWP field trials planted with individual resistant families in replicated designs and assessed for rust damage on relatively young trees are rare (Sniezko et al. 2000, Sniezko et al. 2004a, Hunt 1990, 2002, Goddard et al. 1985, Kinloch et al. 2007). Other types of field plantings of WWP incorporate bulk lots planted in operational field plantings (Bingham et al. 1973, McDonald et al. 1994), or use plantations with a mixture of resistant stock and unimproved stock (Hunt & Meagher 1989), or use transplanted survivors from artificial inoculation trials (Sniezko et al. 2004b, Hunt 2002).

The relationship between spotting frequency and cankering in WWP families is of interest in operational breeding programs to determine the utility of using spotting frequency as an early selection trait (Hunt 2002). The correlation between spotting frequency and percent cankering has been examined in short-term seedling screening trials (Meagher & Hunt 1996, Hoff & McDonald 1980), but there has been little field validation (Hunt 1990). An extension of this relationship that hasn't been examined is the correlation between spotting frequency and the number of cankers.

In this paper, we will focus on one trial with early resistance differences among families on a site with very high infection levels that has been assessed for rust infection and mortality in three consecutive years. We also examine the variation in incidence and severity of cankering among families in the field. Finally we compare field performance of families relative to their performance in a short-term inoculation trial.

Materials and methods

Planting Stock

Seeds from 49 seedling families of western white pine were sown into 'supercell' tubes (164 cm³) in March 1997 at the Dorena Genetic Resource Center (DGRC) in Cottage Grove, Oregon. Of the 49 families, four were half-sibs from phenotypic selections in forest stands, eight were DGRC orchard half-sibs, and 37 were full-sib families (including selfed crosses). A few of the families were advanced generation crosses or backcrosses. The families were derived from 55 unrelated parents (or grandparents), 35 of which were involved in only one cross or open-pollinated seedlot while 20 were involved in two to seven control crosses (table 1). Parents for the control crosses or open-pollinated seedlots were selected in forest stands across Oregon and Washington including: Colville National Forest (1 parent), Gifford Pinochet NF (3 parents), Mount Hood NF (4 parents), Willamette NF (19 parents), Umpqua NF (22 parents), Rogue-Siskiyou NF (5 parents), and Bureau of Land Management Roseburg district (1 parent).

Most of the 49 families planted at Optical were previously evaluated at DGRC in artificially inoculated seedling trials for resistance to white pine blister rust, including 38 in a 1998 trial. For the purpose of this report the families were classified into four categories based on prior seedling screening results:

(1) Cr2: possessing complete resistance due to a hypersensitive response (HR) in needles conditioned by a dominant major gene, Cr2, that prevents further fungal colonization in the stem (Kinlokh et al. 1999).

(2) Q: families with a moderate level of survival in short-term testing but do not have HR. These families may have both stem symptom seedlings and seedlings that survived stem infection (bark reactions or cankers). Many Table 1 Blister rust infection, mortality, and stem symptoms in 49 western white pine families at the Optical field site. Families categorized into resistant groups based on previous short-term test ing: Cr2, Q, Partial Resistance (PR), Susceptible (S): Height (cm) measured in 2002 after five growing seasons. Cumulative percent rust mortality (% Rmort.), stem symptoms (% SS), active infections (% AI), and bark reaction (% BR) through 2004. Number of stem symptoms (# SS), active infections (# AI), and bark reaction (# BR) per tree at the 2004 assessment.

Parents		0	ht Percent					# Stem symptoms		
Female	Male	Group	(cm)	Rmort ^a	SS^b	AIc	BR^d	SS^e	AI^{f}	BR ^g
03014-014 x 03014-016	W	Cr2	85	35	97	100	25	9.6	9.4	0.25
10043-006	15045-837	Cr2	108	6	100	100	36	9.3	9.0	0.26
15045-814 x 15045-837	15045-814 x 15045-837	Cr2	91	13	100	100	16	12.8	12.4	0.40
15045-816	W	Cr2	85	18	97	100	10	7.5	7.4	0.09
15045-816 x 15045-841	W	Cr2	81	19	100	100	3	11.2	11.2	0.03
15045-820 x 06025-510	W	Cr2	78	14	97	97	22	7.5	7.3	0.25
15045-836	W	Cr2	85	24	100	100	29	8.3	7.7	0.63
15045-839	15045-845	Cr2	95	25	100	100	9	13.1	13.0	0.13
15045-840	15045-839	Cr2	82	19	100	100	16	7.6	7.5	0.18
15045-841	15045-851	Cr2	82	6	97	100	16	7.5	7.1	0.38
15045-844 x 15045-862	15045-814 x 15045-837	Cr2	100	24	100	100	4	16.3	15.9	0.38
15045-861	15045-862	Cr2	88	13	100	100	19	17.8	17.4	0.41
15045-862	10043-006	Cr2	84	18	97	100	13	7.2	7.1	0.13
15045-862	15045-837	Cr2	78	23	100	100	9	8.8	8.4	0.31
15045-862	18034-392	Cr2	84	19	100	100	9	15.9	15.8	0.11
15045-862	18034-787 x 18034-787	Cr2	90	33	100	97	41	4.8	3.7	1.02
15045-874 x 15045-896	W	Cr2	79	13	100	100	14	8.0	7.9	0.04
15045-894	15045-820	Cr2	60	22	88	100	6	4.8	4.7	0.04
15045-896	15045-862	Cr2	85	27	97	100	10	14.8	14.6	0.25
15046-894	15045-872	Cr2	89	16	93	100	25	12.6	12.1	0.48
18034-389	18034-390	Cr2	88	14	100	96	11	9.6	9.4	0.13
18034-392	18034-392	Cr2	54	13	100	96	51	6.8	5.8	1.03
18034-395	W	Cr2	78	7	100	100	3	9.1	9.0	0.03
18034-399	18035-395	Cr2	75	6	100	100	13	9.1	8.8	0.28
18035-388	W	Cr2	77	20	96	100	48	10.8	10.0	0.79
B2044-207	15045-820	Cr2	66	13	100	100	6	10.8	10.8	0.07
		Cr2 avg	83	18	98	99	18	10.1	9.7	0.31
06024-506	06024-506	0	79	3	68	63	68	2.8	1.7	1.10
06024-506	06024-504 x 06204-511	ò	80	3	31	71	93	3.3	1.6	1.64
15045-898	15045-879	Q	79	10	54	90	40	6.1	4.8	1.31
18033-704	18033-708	Q	78	3	94	100	28	7.1	6.7	0.32
18033-708	18033-703	Q	77	3	75	100	21	5.2	4.7	0.48
18033-708	18033-704	Q	69	7	72	95	27	4.7	4.0	0.68
		Q avg	77	5	66	87	46	4.9	3.9	0.92
06024-342	W	PR	65	15	96	100	16	71	69	0.20
10043-003	10043-004	PR	85	10	85	100	20	10.0	9.2	0.82
10046-423	10045-020	PR	68	0	93	94	2.5	5.6	53	0.30
10046-423	10046-423	PR	50	6	79	100	19	3.3	3.1	0.21
15045-898	15045-824	PR	75	22	86	84	39	6.0	4.4	1.60
18033-706	18035-412	PR	85	7	97	100	26	7.8	7.6	0.23
18033-707	18034-385	PR	100	14	97	100	22	9.0	8.6	0.35
18034-385	18034-385	PR	66	27	79	100	10	5.6	5.4	0.17
18034-385	18035-412	PR	84	8	96	100	17	7.4	7.0	0.38

Table 1 (continuation	on)
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	Parents	Creen	ht		Perce	ent		# Ste	m syn	ptoms
Female	Male	Group	(cm)	Rmort	¹ SS ^b	AIc	BR	SS ^e	AI^{f}	BR ^g
18034-389	18034-380	PR	773		106	100	25	7.6	7.4	0.19
18034-389	18035-383	PR	85	22	97	100	8	7.6	7.5	0.13
18034-399	18034-389	PR	89	9	97	100	6	10.5	10.4	0.03
18034-399	18035-383	PR	83	4	97	100	11	7.2	7.1	0.16
18035-405	18034-385	PR	115	31	100	100	23	11.2	10.5	0.68
21105-058	W	PR	88	8	100	100	4	11.5	11.5	0.04
		PR avg	81	13	94	99	18	7.8	7.5	0.37
03024-532	W	S	82	34	94	97	26	8.4	8.1	0.29
18035-426	W	S	67	25	100	91	17	6.6	6.0	0.63
		S avg	74	30	97	94	21	7.5	7.0	0.46
		Site Ava	81	15	93	97	22	86	82	0 4 1

^a % Cumulative blister rust mortality = trees dead from rust /[trees alive or dead from rust], (excludes non-rust mort.)

^b % Cumulative stem symptom = rust infected trees / [infected trees + uninfected trees + rust mortality trees], (excludes non-rust mort.)

^c % Cumulative active infection = [trees with normal cankers or partial bark reaction] / trees with stem symptoms

^d % Cumulative bark reaction = tree with bark reaction / trees with stem symptoms

^e # stem symptoms = average # SS (AI or BR) for living trees with stem symptoms in 2004

f # active infections= average # AI cankers for living trees with stem symptoms (AI or BR) in 2004

^g # bark reactions = average # BR for living trees with stem symptoms (AI or BR) in 2004

seedlings in these families also manifest stem symptoms a year later than the susceptible control families, and mortality is often at least one year later (Sniezko & Kegley 2003).

(3) PR: families with lower levels of partial resistance in short-term testing than Q families. These families generally have a high percentage of trees with stem symptoms but may have a low frequency of canker-free trees, trees with bark reaction or trees that survive with cankers.

(4) S: susceptible control families with little or no documented resistant responses and very high to 100% mortality in short-term screening trials.

The Cr2 group included 26 families with at least one parent confirmed to have the Cr2gene based on seedling screening data from DGRC (unpublished data) or the Institute of Forest Genetics (Kinloch et al. 1999). A few half-sib families produced in areas where Cr2is likely the predominant pollen source (e.g. DGRC orchards) were also included in this group. The Cr2 group was composed of F_2 and F_1 full-sib families, half-sib families from forest selections and orchard trees. The majority of the families are heterozygous for the *Cr2* gene and the parents were selected from Champion Mine and Grouse Mountain on the Umpqua NF, or Bear Pass and surrounding areas on the Willamette NF.

The 'Q' group was composed of six full-sib families produced from control crosses amongst seven parents. The 'PR' group was composed of 15 families with an array of putative partial resistant mechanisms (e.g. bark reaction, slow canker growth, and tolerance). The PR group contained 13 full-sib families and two half-sibs collected from forest stands. The susceptible group included two half-sib families from forest stands, and both showed a high incidence of infection and mortality in previous screening trials.

Planting Sites

The seedling families were planted at three field sites, but only the 'Optical' site, had

enough rust infection by the 2004 assessment to report on here. *Ribes sanguinium* (an alternate host of *C. ribicola*) occur naturally throughout the planting site. Previous sampling of the rust has shown a moderate incidence of the virulent race of the rust (*vcr2*) that overcomes HR resistance is present in much of this local area (Kinlokh et al. 2004).

Optical was logged and broadcast burned in 1997, and soil compaction was minimal. The area surrounding Optical was replanted with mostly *Pseudotsuga menziesii*. The field trial occupies approximately four acres and was planted by hand using shovels in May 1998.

The Optical trial included 1,556 trees in a random-complete block design (RCB) with eight blocks. Families were arranged into row-plots randomized within blocks. Family row-plots contained three to four seedlings.

Field Assessments

The Optical site was assessed for mortality in 2000. Blister rust was not found on the trees at this time. The first rust assessment was taken in 2002 after it was apparent that a moderate level of stem infection was present. Additional rust assessments were made in 2003 and 2004. At each assessment tree height was measured and stem infections were counted on the bole and branches. Stem infections were classified as normal canker (NC), partial bark reaction (PBR), or bark reaction (BR). A normal canker is initially manifested as a small orange discoloration of the bark, and as it develops it sometimes displays a classic diamond shape or fusiform swelling of the bark with an active orange margin (Kinlokh & Davis 1996, Hunt 1997). A bark reaction is an incompatible interaction between the fungus and the bark tissue forming a sunken necrotic lesion on the stem. When no fungus activity is observed, the *BR* is considered 'complete' while a partial bark reaction (PBR) does not completely halt fungal growth (Kegley & Sniezko 2004 and references therein). In this report a bark reaction refers to the 'complete' BR, and NC and PBR were classified as active infections (AI). Trees can have a multitude of stem symptoms (SS), and the total number of SS can change over time, as can the relative proportion of BR and AI. Most stem symptoms in the field are visible one to three years after needle infection.

In addition, trees were rated for damage, severity, and vigor using a classification system. Tree 'vigor', relating to tree growth and live/dead status, was classified on each tree as either: alive and vigorous, alive and sickly, recently dead, dead more than a few years, dead or missing (unable to determine presence of rust), or top-dead from rust. Tree vigor gives a rating of tree health to distinguish families where there may be little impact on growth despite being infected. Sources of damage were classified to distinguish blister rust damage from damage caused by other diseases, insects, climate, etc. The severity of damage was coded as slight, moderate, severe, broken top, dead, or none.

Seedling inoculation screening trial

A separate set of seedlings from 38 of the 49 families planted at Optical were artificially inoculated in 1998 at DGRC. This report will only address a few traits examined in the families common to the field and the seedling inoculation trials. Seedlings were grown outside in boxes for two growing seasons before being moved into an inoculation chamber. Each box contained 12 families replicated six times in a RCB design with 10-tree row plots. Wire racks were placed above the planting boxes and Ribes leaves infected with Cronartium ribicola at the telia stage were placed on the racks. The Ribes were collected from forested areas in Oregon and Washington where vcr2 was absent or in very low frequencies (Kinloch et al. 2004). The seedlings were inoculated at 17°C and 100% humidity for 10 hours reaching an average inoculum density of 3,000 basidio spores/cm². At this time the *Ribes* leaves were removed, and the trees remained in the chamber for 48 hours at favorable conditions for spore germination and needle infection (19°C and 100% humidity). Spore germination was estimated at 99% for this trial. Kegley & Sniezko (2004), Sniezko (1996) and Samman (1982) provide more details of the inoculation procedure at DGRC.

The seedling trial was assessed six times

over five years. Traits assessed included: number of needle lesions in the first year, and for each additional assessment, presence or absence of stem symptoms, type of stem symptom (BR or NC), and whether alive or dead from rust or other causes. Bark reaction was scored if the seedling exhibited a complete bark reaction or a partial bark reaction. The numbers of stem symptoms were not counted in this trial. An estimate of the number of needle lesions ('spot') per seedling was obtained by using spot classes determined from monitoring control families. The spot classes were distributed as follows: Class 1, 1-3 spots; Class 2, 4-9 spots; Class 3, 10-22 spots; and Class 4, >22 spots. The spotting incidence in this trial represents a moderate level of spots at DGRC (unpublished data).

Data Analysis

The traits used to summarize the incidence of blister rust resistance in the field trial included percent blister rust mortality (Rmort), stem symptom (SS), active infection (AI), and bark reaction (*BR*). For this paper, active infections included both normal cankers and partial bark reactions. In addition the average number of SS, AI, and BR were calculated for infected seedlings in the 2004 assessment. These characters are downward biased because canker counts from trees dying on or before the 2004 assessment weren't included. A higher percentage of these censored trees were in the susceptible controls (table 1). Mean family height in centimeters from the 2002 assessment was reported as an indication of tree size after infection, but prior to the occurrence of most severe rust damage. Details of the character calculations are in table 1.

A subset of traits traditionally calculated in seedling screening trials at DGRC for the last 20 years (Kegley & Sniezko 2004, Sniezko & Kegley 2003) were used to compare family performance in the 1998 seedling screening trial with field performance. The traits used for this report included: mean family height (*HT3*) after three growing seasons (1 year after inoculation), mean needle lesion class, percent stem symptom (*SS*), percent bark reaction (*BR*), and percent infected (*SS* or needle spots) that were alive five 5 years after inoculation (*RSurv5*). A composite trait, Q, (percent of trees stem symptom free or having bark reaction) was also included in the analysis (c.f. Sniezko & Kegley 2003 for computational details).

Family means were calculated from plot means. The overall trial means were calculated from family means. Pearson correlations of family means were calculated using PROC CORR procedure in SAS version 9.1.3 with the critical p - value determined using the conservative Bonferroni method to control the group error rate for multiple statistical tests (RICE 1989). The critical p - value for the correlations among traits at Optical was p <0.0024 for 21 correlations. The critical p value for the correlations between the 1998 seedling screening trial and Optical was p <0.0021 for 24 correlations. The relationship between mean needle lesion in screening and the number of cankers in field was treated as an a priori test and evaluated at the p < 0.05level.

Results

Optical field results

Post-planting mortality (non-rust related mortality) assessed in 2000 (two years after planting) was nine percent and reached a plateau at 11% in 2003. At the first assessment in 2002, after potentially four seasons exposure to blister rust, 70% of trees had stem symptoms and the number of stem symptoms per family ranged from two to six. The families in the Q group had the lowest incidence of infection ranging between 13 and 34%. Blister rust mortality was less than one percent, occurring in only a few families, but 14% of living trees had 'severe damage' from one or more large cankers on the bole. Height in 2002, representing growth before the most severe blister rust damage occurred, averaged 81 cm.

The incidence and severity of stem symptoms (SS) increased substantially from 2002 to 2004. At the 2004 assessment 93% of the trees had SS, but some differentiation among families was evident with family means ranging between 31 and 100% SS (table 1). Families in the Q group had on average lower SS (66%) while the *PR*, *Cr2*, and S groups averaged 94-98 % (table 1, figure 1). There was a large range among families for number of stem symptoms (*SS*) per tree (2.8 to 17.8). Families in the Q resistance group averaged fewer *SS* (4.9); the *PR* and S groups were intermediate with 7.8 and 7.5, respectively, and the *Cr2* group averaged 10.1 stem symptoms (table 1). As expected the families in the *Cr2* group with HR resistance showed high infection levels in the presence of the *vcr2* race of rust at Optical.

The average number of SS in infected seedlings was positively correlated with percent SS (table 2, figure 2). The Q families had lower percent SS and the seedlings that were infected had fewer numbers of SS. In contrast, the majority of seedlings in the Cr2 families were infected and they averaged high numbers of SS (table 1). At this point the correlation between percent SS and percent rust mortality (Rmort) was not significant (table 2).

All families exhibited both active infections (*AI*), from normal cankers or incomplete bark reactions, and bark reactions (*BR*) but the inci-





dence of these traits varied by family. The incidence of AI across the 49 families was 97%, with family means ranging from 63 to 100%. The incidence of BR was 22%, with family means varying from 3 to 93% (table 1). One outstanding family from Mt. Hood NF had only 31% of trees with stem infections and 93% of these infected trees had one or more bark reactions.

Percent AI and BR amongst the 49 families was negatively correlated (table 2). Families in the Q group had higher percentage of BR and relatively low percentage of AI, while families in the other resistance groups tended to have low BR and high AI percentage (at or near 100%) (table 1, figure 1). The correlation between percent AI and BR was also significant when computed separately for the 26 Cr2 families and 23 non-Cr2 families (PR, Q, S), however the relationship was stronger among the non-Cr2 families (data not shown). Percent AI was positively correlated with percent SS, whereas percent BR had a negative correlation with percent SS (table 2), indicating that families with high percentage BR and low percentage AI had relatively low percent infection. At the low level of mortality occurring through 2004, the correlation between percent mortality and percent AI or percent BR were not significant (table 2).

The mean number of AI and BR, was 8.2 (family range; 1.6 to 17.4) and 0.41 (family range; 0.03 to 1.6) respectively (table 1). Only 26 trees (2%) had bark reactions and no other



Figure 2 Relationship between percent stem symptom and number stem symptoms per tree for 49 families at Optical site. Pearson correlation r = 0.51, p < 0.001.

Table 2 Correlations among 49 families at the Optical field site. All pairwise comparisons among: average number stem symptoms (#*SS*), active infections (# *AI*), bark reactions (# *BR*) per tree at the 2004 assessment; and cumulative percent stem symptoms (% *SS*), rust mortality (% *Rmort.*), active infections (% *AI*), and bark reaction (% *BR*) through 2004. Pearson's correla tion coefficient (r) and significance level (p -value). Significance evaluated using a Bonfer roni correction at p = 0.0024 for 21 correlations. Significant correlations in bold

Trait/trait	# SS	# AI	# BR	%SS	% RMORT	% AI	%BR
#SS	-		-	-	-		-
# AI	0.99***		-	-	-	-	-
# BR	-0.34	-0.44**	-	_	-	_	-
%SS	0.51***	0.56***	-0.63***	-	-	-	-
% RMORT	0.27	0.26	-0.02	0.29	-	-	-
% AI	0.45^{**}	0.51***	-0.70***	0.69***	0.21	-	-
%BR	-0.44**	-0.52***	0.82***	-0.64***	-0.19	-0.76***	-
* p < 0.01 **	p < 0.0024	*** <i>p</i> < 0.001					

active infections. These 26 trees averaged 2.3 bark reactions per tree and were distributed among 12 families including four of the six Q families (data not shown). In addition, crosses with the parent 06024-506 from the Mt. Hood NF had a higher incidence of trees with only *BR*. A self cross of this parent resulted in 25% *BR* only trees.

Families in the Q resistance group averaged higher number BR and lower AI than the PR, Cr2, and S groups (table 1). Since bark reactions were relatively rare the total number of stem symptoms was mostly influenced by the number of active infections (# SS = AI + BR). Thus the number AI had a high correlation with number SS (table 2) and both traits had a similar magnitude and pattern amongst the resistant groups. The PR, Cr2, and S groups had more than twice the number of SS and AI compared with the Q group (table 1). Not surprisingly, both traits have a similar correlation structure with the other traits (table 2). That is, both the number of SS and AI were positively correlated with percent SS and AI, and negatively correlated with percent BR. In contrast, number BR was negatively correlated with percent SS and AI, and positively correlated with percent BR. Finally, the number of BR was correlated with number AI but not significantly correlated with number SS using the Bonferroni correction. At this time the correlations between percent rust mortality and number of SS, AI, and BR were not significant.

Blister rust mortality increased to 15% by

2004, and the family mortality varied between 0 and 35%. As a group the Q families had the lowest mortality (5%), the *PR* and *Cr2* families were intermediate, and the susceptible control families were highest (30%) (table 1, figures 1 & 2). Although blister rust mortality was at its early stages in 2004, it was notable that the *Cr2* families had lower mortality than the susceptible control families despite the *Cr2* families having higher percent stem symptoms and number of stem symptoms (table 1). The incidence of severe damage from large basal cankers or top-dead from rust in 2004 was 24% (data not shown); most of these trees are expected to die from rust in the near future.

Comparison of seedling testing and field results

38 of the 49 families planted at Optical were inoculated in 1998 in a seedling screening trial at DGRC. For the 36 'resistant' families in the 1998 trial, 98% of the seedlings had needle lesions (spots), 63% had stem symptoms, and rust survival was 39%. The two susceptible control families averaged 97% spotting, 93% stem symptoms, and 5.8% rust survival. The *Cr2* and Q families had similar trait values for percent *SS* and *Rsurv5* but Q families had higher percentage *BR* and seedlings in spot classes 0-2. The susceptible control families were rated poor for the same traits (data not shown).

Family height was correlated between the

seedling screening trial and the Optical field site (r = 0.52, p < 0.001). Cr2 and PR families tended to be taller than the Q and susceptible control families at Optical (table 1) and in the 1998 seedling screening trial at DGRC (data not shown). The parents of Cr2 and PR families were selected in stands closer to the Optical planting and DGRC, whereas parents in the Q and S families were from more northern areas. Family height was correlated with number AI and SS, but not with number BR or percent SS, AI, and BR at Optical. Similarly, family height in the DGRC trial was also not correlated with percent SS or BR in the 1998 seedling screening trial (data not shown). The absolute number of SS was potentially influenced by tree height as taller families had more infection sites (or needles), but other genetic and gene x environment factors were confounded. For instance the Cr2 families had the highest number of SS and were the tallest families. However they were growing in an environment similar to their local environment, and they were the most susceptible at Optical due to the presence of the *vcr2* strain of rust overcoming the *HR* resistance.

The correlation between percent SS at DGRC and the Optical field site was low (r =0.002, p = 0.99) across all 38 families. The low correlation was not unexpected due to the interaction between the 18 Cr2 families and the different rust races present in the two trials. The 1998 seedling screening trial used wildtype inoculum from geographic sources with *vcr2* absent or in very low frequency, while the *vcr2* race of rust is present at Optical. As a result, the Cr2 families at DGRC had levels of stem symptoms (43%) consistent with a dominant major resistant gene, whereas percentage of SS in the Cr2 families averaged 98% at Optical as the *vcr2* race of rust overcame the Cr2 gene. The S and PR families had high percent SS in both trials, and the Q families had relatively lower percent SS at DGRC (55%) and Optical (66%). The correlation between percent SS in screening and field increases substantially (r = 0.71) when the 18 families with Cr2 were omitted (table 3, figure 3).

Due to the relative susceptibility of the Cr2 families at Optical, they were excluded from many of the remaining comparisons; or in some cases results will be presented separately

for the 20 non-Cr2 and 18 Cr2 families. Percent SS in the 20 non-Cr2 families in the DGRC trial was positively correlated to number of SS and percent SS, and negatively correlated with percent BR at Optical. There was a non-significant trend between percent SS in the DGRC trial with number BR, percent Rmort, and percent AI at Optical using the conservative Bonferroni correction (table 3). Thus families with the lowest percent SS in the DGRC inoculation tended to have fewer SS, lower percent AI and SS, higher percent BR, and more bark reactions at Optical.

In the 1998 seedling screening at DGRC, 98% of the seedlings had needle lesions (spots) and family mean needle lesion per group (e.g. Cr2, Q, etc) was similar (data not shown). The mean needle lesion class for P. monticola in the 1998 trial was 2.4, which is a moderate level of spotting in DGRC inoculations (data not shown). There was a moderate relationship between family needle lesion frequency in seedling testing and number of SS at Optical across the 38 common families (r = 0.42, p =0.01, figure 4). The relationship was also significant for the 20 non-Cr2 families (r = 0.49, p = 0.03) suggesting the needle lesion trait assessed in short term seedling screening trials may be a useful predictor of the frequen-



Figure 3 Relationship between percent stem symptoms at Optical field site and seedling screening at Dorena GRC for 20 non-*Cr2* families. Data summarized though six years exposure to rust at Opti cal and five years after inoculation at DGRC. Pearson correlation r = 0.71, p < 0.001

Table 3 Pearson correlations between traits at the Optical field site and seedling screening in the 1998 trial at Dorena GRC. Optical traits include: average number stem symptoms (# SS) and bark reaction (# BR) per tree; and percent stem symptom (% SS), rust mortality (% Rmort), active infections (% AI), and bark reaction (% BR). Artificial screening traits include: percent bark reaction (% BR), stem symptoms (% SS), percent rust survival 5 years post inoculation (% RSurv5), and percent Q (% Q). Pearson's correlation coefficient (r) and significance level (p - value). Significance value evaluated using a Bonferroni correction at p = 0.0021 for 24 correlations. Significant correlations in bold.

	Optical Field Site								
DGRC 98	# SS	#BR	% SS	% RMORT	% AL	% BR			
% BR ^a	-0.52***	0.74***	-0.72***	-0.28	-0.66***	0.76***			
% SS ^b	0.69***	-0.53	0.71***	0.53	0.63*	-0.69***			
% RSURV ^b	-0.56*	0.67^{**}	-0.79***	-0.47	-0.60*	0.73***			
% Q ^b	-0.55	0.75**	-0.77***	-0.43	-0.63*	0.76***			

* p < 0.01 ** p < 0.0021 *** p < 0.001

^a38 families common between DGRC seedling screening and Optical field site

^b20 non-Cr2 families common between DGRC seedling screening and Optical field site

cy of stem symptoms in the field for non-Cr2 families. However, the correlation was low and non-significant among the 18 Cr2 families (r = 0.13, p = 0.61).

Percent bark reaction in the DGRC trial was positively correlated with percent BR at Optical (figure 5) and number of BR at Optical across the 38 common families (table 3). Percent BR in the DGRC trial incorporated both complete *BRs* and PBRs whereas *BR* at Optical included only complete BR. Similar to the Optical results, the Q families had the highest BR percentage and the Cr2 had the lowest in the seedling trial (data not shown). In addition, percent BR in seedling screening was negatively correlated to percent AI, percent SS, and number SS; and positively correlated with number BR at Optical (table 3). These correlations were influenced by two groups of families. The O families had higher percent BR in the DGRC trial, moderate percent AI and SS at Optical, and fewer numbers of SS at Optical. The PR, Cr2, and S groups tended to have lower percent BR in screening, percent AI and SS around 90-100% at Optical, and greater numbers of SS at Optical.

The composite Q trait, calculated as percentage of seedlings that had bark reaction or no stem symptoms, among the 20 non-Cr2 families in the 1998 DGRC trial was negatively correlated with percent SS and positively correlated with percent BR at Optical (table 3). This was the first confirmation that the Q trait determined in short-term screening also had similar characteristics in a field setting. The better performing families in both trials have a combination of both higher incidence of stem symptom free and bark reaction individuals.

Percent rust survival in screening after the 5th year (*RSurv5*) was negatively correlated with percent SS and positively correlated with percent *BR* and number *BR* in the 20 non-*Cr2* families (table 3). Compared with the Q families, the *PR* and S families had lower survival in the DGRC trial with higher percent *SS* and lower percent *BR* and fewer number *BR* at Optical (table 1). There was non-significant trend for a negative correlation between *RSurv5* at DGRC and number of *SS* and percent *AI* at Optical. At this early stage of field mortality the correlation between *RSurv5* at DGRC and Rmort at Optical was non-significant (table 3).

Discussions

Large family variation in field resistance has been demonstrated at several other sites (Sniezko et al. 2004a, Kinloch et al. 2007), however this trial had the largest number of families, the highest rust infection level, and a high infection from *vcr2*, a virulent race of rust that overcomes one of the resistant mechanisms (HR). The high infection level presents an opportunity to evaluate the effectiveness of



Figure 4 Relationship between number stem symptoms at Optical field site and mean needle lesion class in seedling screening at Dorena GRC for 38 families. Data summarized though six years exposure to rust at Optical and needle lesions 9months after inoculation. Midpoint of lesion class (# lesions): class 1 = 2, class 2 = 6.5, class 3 = 16, class 4 = > 22. Pearson correlation r = 0.42, p = 0.01

several types of resistance under high disease pressure.

Many of the resistant responses (e.g. bark reaction) selected for in DGRC screening trials occur at relatively low levels (10 to 25%) after initial stem infection, so the high level of stem infection in many families at this high hazard site are not unexpected. In the case of Optical, the partial resistant families had similar levels of resistance as the susceptible control families including a high percentage of SS and AI, low percentage of BR, moderate number of stem symptoms and bark reactions. However, at this early stage of mortality at Optical the PR families had half the level of mortality as the susceptible control families. For the *PR* families, our main interest lies in resistant responses after stem infection and the rate of survival. The assessments over the next five years should provide further needed information.

As in testing of non-Cr2 families at Dorena (Sniezko & Kegley 2003, Kegley & Sniezko 2004) the most resistant families in this field trial tend to show an array of attributes: fewer stem symptoms, lower percentage of trees infected, lower percentage of active cankers, and higher incidence and frequency of bark







reactions. The Q families were rated the most resistant for these traits at the Optical field site. The resistant responses in Q families have been characterized at the phenotype level but the underlying mechanism(s) are still unknown (Sniezko & Kegley 2003); however the phenotypic data suggests some mechanism prevents needle infections from spreading into the stem, and slows or stops the progression of stem infections that do occur.

The Cr2 families were primarily selected for *HR*, and in the presence of *vcr2* at this field trial they had the highest number of stem symptoms of any group and performed similarly to the susceptible control families on other traits including a high percentage of trees with stem symptoms. However, early mortality is delayed relative to the susceptible control families suggesting some form of slow rusting resistance may be present. Future monitoring of mortality will help clarify this resistance response and determine if the Cr2 families have any other partial resistance beyond Rgene resistance. WWP families with Cr2 resistance in other field sites was shown to be effective for over 20 years, but the presence of *vcr2* in later years has increased the level of infection and mortality (Kinloch et al. 2007).

Early performance of WWP families in a natural field setting are tracking reasonably well with expectation from screening trials at DGRC including percent stem infection and bark reaction traits. Hunt (1990) also found a good correspondence between percent cankering in inoculation trials and field plantations for WWP families in British Columbia. The moderate correspondence between needle lesion frequency recorded within one year after inoculation and number of the stem symptoms after potentially 6 years' exposure to rust at a field setting suggests spotting frequency in short-term screening may be a useful predictor of the severity of cankering in the field. Hunt (1990) did not find a significant correlation between spotting frequency and the percent cankers in the field using a smaller sample size than the current study. It was speculated that a narrow range in spotting counts was influencing the results in the Hunt (1990) study.

Family variation in rust resistance is clearly evident at this site at this early age. These results are encouraging and seem to correspond relatively well to the infection levels in the inoculation test of seedlings. However, further time is needed to evaluate differences in survival among the infected trees in the field test and how this relates to the seedling test. Moderate levels of mortality from existing cankers are expected to occur soon, and there will likely be additional new infections in the ensuing years.

The selection and breeding program for resistance in western white pine is only in the early stages. Large base populations have been established, but relatively little breeding work has been done using the initial selections from resistance screening. The Optical site appears to represent a moderately high rust hazard and will be a good test of resistance under these conditions. Many additional field sites have been established in the last 10 years in Oregon and Washington. Long-term monitoring of this trial and others will help determine durability of resistance, and which types of resistance will be useful under different rust hazard conditions. Subsequent breeding efforts or modifications to screening protocols will be influenced by results from this and other field trials.

References

- Fins, L., Byler, J., Ferguson, D., Harvey, A., Mahalovich, M.F., McDonald, G., Miller, D., Schwandt, J. & Zack, A. 2002. Return of the giants: restoring western white pine to the inland northwest. J. Forestry. 100(4): 20-26.
- Goddard, R.E., McDonald, G.I. & Steinhoff, R.J. 1985. Measurement of field resistance, rust hazard, and deployment of blister rust-resistant western white pine. USDA Forest Service, Intermountain Research Station Research Paper. INT-358. 8 pp.
- Hoff, R., Bingham, R.T. & McDonald, G.I. 1980. Relative blister rust resistance of white pines. Eur. J. For. Path. 10: 307-316.
- Hoff, R.J. & McDonald, G.I. 1980. Resistance to *Cronartium ribicola* in *Pinus monticola*: reduced needle-spot frequency. Can. J. Bot. 58: 574-577.
- Hunt, R.S. 2002. Relationship between early family-selec tion traits and natural blister rust cankering in western white pine families. Can. J. Plant Pathol. 24: 200-204.
- Hunt, R.S. 1997. Relative value of slow-canker growth and bark reactions as resistance responses to white pine blister rust. Can. J. Plant Pathol. 19: 352-357.
- Hunt, R.S. 1990. Blister rust in inoculated and plantationtested western white pine in British Columbia. Can. J. Plant Pathol. 12: 279-282.
- Hunt, R.S. & Maegher, M.D. 1989. Incidence of blister rust on 'resistant' white pine (*Pinus monticola* and *P. Strobus*) in coastal British Columbia plantations. Can. J. Plant Pathol. 11: 419-423.
- KEGLEY, A.J. & SNIEZKO, R.A. 2004. Variation in blis ter rust resistance among 226 *Pinus monticola* and 217 *P. lambertiana* seedling families in the Pacific Northwest. In: Breeding and genetic resources of fiveneedle pines: genetics, breeding, and adaptability. Proceedings of the IUFRO 2.02.15 Working Party Conference (eds. R.A. Sniezko, S. Samman, S.E. Schlarbaum & H.B. Kriebel). Proceedings RMRS-P-32. USDA Forest Service Rocky Mountain Research Station, Ft. Collins, CO. pp. 209-226.
- King, J.N. & Hunt, R.S. 2004. Five needle pines in British Columbia, Canada: past, present and future. In: Breeding and genetic resources of five-needle pines: genetics, breeding, and adaptability. Proceedings of the IUFRO 2.02.15 Working Party Conference (eds. R.A. Sniezko, S. Samman, S.E. Schlarbaum & H.B. Kriebel). Proceedings RMRS-P-32. USDA Forest Service Rocky Mountain Research Station, Ft. Collins, CO. pp. 12-19.
- Kinloch, B.B., JR., Davis, D.A. & Burton, D. 2007. Resis tance and virulence interactions between two white pine species and blister rust in a 30-year field trial. Tree Genetics and Genomes Online First: 10 p.
- Kinloch, B.B., JR., Sniezko, R.A. & Dupper, G.E. 2004. Virulence gene distribution and dynamics of the white pine blister rust pathogen in western North America. Phytopath. 94: 751-758.
- Kinloch, B.B., JR., Sniezko, R.A. & Dupper, G.E. 2003. Origin and distribution of *Cr2*, a gene for resistance to

white pine blister rust in natural populations of western white pine. Phytopath. 93: 691-694.

- Kinloch, B.B., JR., Sniezko, R.A., Barnes, G.D. & Greathouse, T.E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade range. Phytopath. 89: 861-867.
- Kinloch, B.B., JR. & Davis, D.D. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine. In Sugar pine: status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee (eds. B.B. Kinloch Jr., M. Marosy & M.E. Huddleston). Univ. Calif. Div. Agr. Nat. Res. Publ. 3362. pp. 125-132.
- Meagher, M.D. & Hunt, R.S. 1996. Heritability and gain of reduced spotting vs. blister rust on western white pine in British Columbia, Canada. Silv. Genetica 45: 2-3.
- McDonald, G.I. & Hoff, R.J. 2001. Blister rust: An introduced plague. In: Whitebark Pine Communities. (ed. D.F. Tomback). pp.193-220. Washington, D.C. Island Press.
- McDonald, G.I., Hoff, R.J., Rice, T.M. & Mathiasen, R. 1994. Measuring early performance of second generation resistance to blister rust in western white pine. In: symposium proceedings: Interior cedar-hemlock-white pine forests: ecology and management. Pullman, Washington, USA. pp. 133-150.
- McDonald, G., Zambino, P. & Sniezko, R.A. 2004. Breed ing rust-resistant five-needle pines in the western United States: lessons from the past and a look to the future. In: Breeding and genetic resources of five-needle pines: genetics, breeding, and adaptability. Proceedings of the IUFRO 2.02.15 Working Party Conference (eds. R.A. Sniezko, S. Samman, S.E. Schlarbaum & H.B. Kriebel). Proceedings RMRS-P-32. USDA Forest Service Rocky Mountain Research Station, Ft. Collins, CO. pp. 28-50.
- Rice, W.R. 1989. Analyzing tables of statistical tests. Evo lution 43(1): 223-225.
- Samman, S.A. & Kitzmiller, J.H. 1996. The sugar pine program for development of resistance to blister rust in the pacific southwest region. In: Sugar pine: status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee (eds. B.B. Kinloch, Jr., M. Marosy & M.E. Huddleston). Univ. Calif. Div. Agr. Nat. Res. Publ. 3362. pp. 162-170.
- Samman, S.A. 1982. The white pine blister rust program of Region 6. In: Breeding insects and disease resistant forest trees. Proceedings, Servicewide genetics workshop, Eugene, Oregon, USA. US. Department of Agriculture, Forest Service. pp. 133-183.
- Sniezko, R.A. 1996. Developing resistance to white pine blister rust in sugar pines in Oregon. In: Sugar pine: status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee (eds. B.B. Kinloch Jr., M. Marosy & M.E. Huddleston). Univ. Calif. Div. Agr. Nat. Res. Publ. 3362. pp. 171-178.

- Sniezko, R.A., Bower, A. & Danielson, J. 2000. A comparison of early field results of white pine blister rust resistance in sugar pine and western white pine. HortTechnology 10(3): 519-522.
- Sniezko, R.A. & Kegley, A.J. 2003. Blister rust resistance experiences in Oregon/Washington: evolving perspectives. In: Proceedings of the Fiftieth Western International Forest Disease Work Conference (comps. H. Maffei & J.M. Stone). USDA Forest Service Central Oregon Forest Insect and Disease Center, Bend, OR. pp. 111-117.
- Sniezko, R.A., Bower, A.D. & Kegley, A.J. 2004a. Variation in *Cronartium ribicola* field resistance among 13 *Pinus monticola* and 12 *P. lambertiana* families: early results from Happy Camp. In: Breeding and genetic resources of five-needle pines: genetics, breeding, and adaptability. Proceedings of the IUFRO 2.02.15 Working Party Conference (eds. R.A. Sniezko, S. Samman, S.E. Schlarbaum & H.B. Kriebel). Proceedings RMRS-P-32. USDA Forest Service Rocky Mountain Research Station, Ft. Collins, CO. pp. 203-208.
- Sniezko, R.A., Kinloch, G.G., Bower, A.D., Danchok, R.S., Linn, J.M. & Kegley, A.J. 2004b. Field resistance to *Cronartium ribicola* in full-sib families of *Pinus monticola* in Oregon. In: Breeding and genetic resources of five-needle pines: genetics, breeding, and adaptability. Proceedings of the IUFRO 2.02.15 Working Party Conference (eds. R.A. Sniezko, S. Samman, S.E. Schlarbaum & H.B. Kriebel). Proceedings RMRS-P-32. USDA Forest Service Rocky Mountain Research Station, Ft. Collins, CO. pp. 243-249.

Rezumat. Kolpak S.E., Sniezko R.A., Kegley A.J., 2008. Inocularea cu rugină veziculoasă și supraviețuirea în teren a 49 familii de *Pinus monticola* la șase ani după plantare. Ann. For. Res. 51: 67-80.

Experimentul din teren evaluează rezistența la rugina veziculoasă a 49 familii originare din statele Oregon și Washington. Site-ul Optic a arătat că numărul arborilor infectați la șase ani după plantare a fost de 93%, însă în prezent mortalitatea atribuibilă ruginii este scăzută. Intervalul de variație a familiilor infectate a fost de cuprins între 31 și 100% iar numărul simptomelor a fost cuprins între trei și 18. După cum a evidențiat Site-ul Optic, familiile purtătoare a Genei-R de rezistență (HR) aparținând la Cr2 au manifestat susceptibilitate ridicată iar acest fapt se datorează prezenței unei rase virulente de rugină vcr2. Familiile fără gena Cr2 posedând o frecvență ridicată de arbori fără atac de cancer sau fără simptomul reacție de scoarță (Familiile 'Q') urmare infecției artificiale efectuată la Centrul de Resurse Genetice de la Dorena (DGRC) (Oregon), au fost cele mai rezistente potrivit Site-ului Optic etalând o mai mare frecvență a mecanismului reacție de scoarță și procente mai mici de arbori infectați, de arbori uscați precum și un număr mai mic de simptome

pe tulpină. Puieții aparținând unui alt set mai mic de familii a fost inoculat la DGRC folosind rase de rugină care nu aparțineau rasei virulente de rugină vcr2. Familiile Cr2 au manifestat un nivel de rezistență previzibil, de asemenea și Familiile 'Q' au etalat nivele de rezistență în testele cu puieți efectuate la DGRC. Au fost puse în evidență corelații pozitive între caracterele simptom pe tulpină și reacție de scoarță exprimate în procente, pe deoparte, și inoculările artificiale controlate și cele naturale din teren, pe de altă parte. De asemenea caracterul numărul de leziuni pe ace evaluat în inoculările artificiale s-a corelat pozitiv cu numărul de simptome pe tulpină observat la Site-ul Optic.

Cuvinte cheie: *Pinus monticola, Cronartium ribicola,* rugina veziculoasă a pinului alb, *Gena-R* de rezistență, *reacția de scoarță*, teste de câmp. (Tradus de I. Blada)

(114440) 40 1. 2144