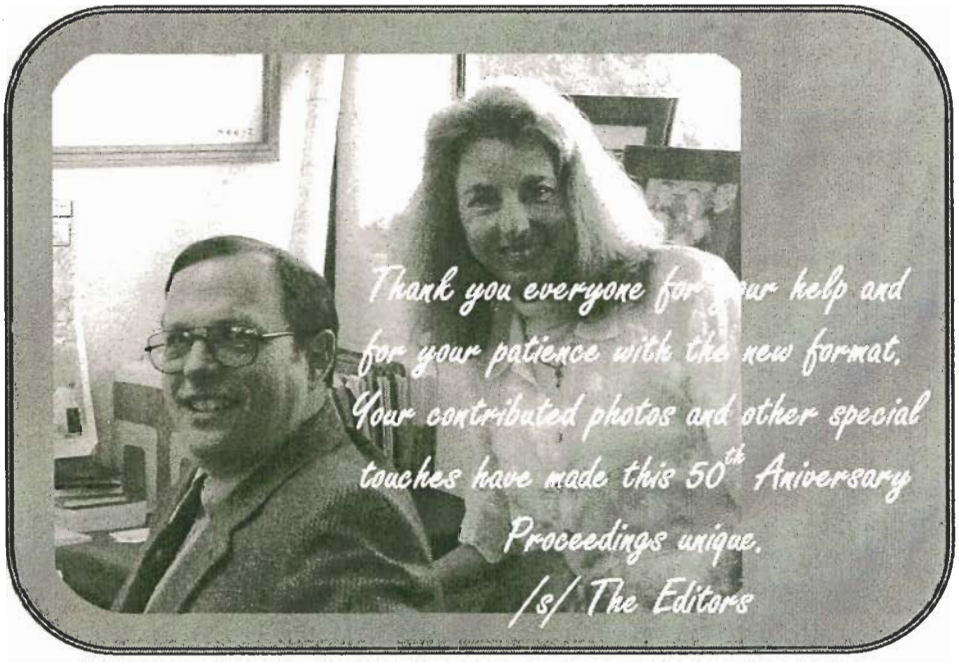
Proceedings Of The Fiftieth Western International Forest Disease Work Conference

Coast Town Centre Hotel
Powell River, BC

OCTOBER 7-11, 2002



COMPILED BY:

Jim Stone and Helen Maffei
USDA Forest Service
Central Oregon Forest Insect and Disease Service Center
1641 Hwy 20 East
Bend, Oregon 97701
April 2003

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Blister Rust Resistance Experiences in Oregon/Washington: Evolving Perspectives

Richard A. Sniezko and Angelia Kegley

Summary

Throughout much of western North America, populations of five-needle pines have been dramatically reduced in natural ecosystems by white pine blister rust, caused by an introduced pathogen, *Cronartium ribicola*. In Oregon and Washington, the resistance program has evaluated progenies of over 4900 western white pine (WWP) and 4500 sugar pine (SP) phenotypic selections since its inception in the late 1950's. Rust screening has shown that while progenies of most selected parents are susceptible, major gene resistance and forms of partial resistance are present. Resistance mechanisms occur at low frequencies, and at least one type, a hypersensitive reaction in the needles of WWP and SP, appears to be geographically restricted. Another newly delineated type of complete resistance, Mechanism 'X' (40-60% of half-sib seedlings from selected parents free of stem symptoms following artificial inoculation), may be present in WWP in very low frequency (<1%) among phenotypic selections. Another form of resistance (Mechanism 'Q') includes both a high proportion of stem-symptom-free and bark reaction individuals within a half-sib family. The underlying bases of these resistances (Mechanisms X and Q) and their relations to other observed resistance responses are unknown but may represent a continuum of resistance response that may include latent development of stem symptoms, delayed mortality, or tolerance. Field trials have been established in Oregon, Washington, and California to validate performance of selected WWP and SP families. Early results from these field trials are encouraging.

Introduction

The introduced disease white pine blister rust, caused by *Cronartium ribicola* J.C. Fisch., has caused heavy mortality of five-needle pines in western North America. Resistance breeding programs for western white pine (*Pinus monticola* D. Don) and sugar pine (*P. lambertiana* Dougl.) were started in the 1950s and have supplied seed for restoration and reforestation efforts. The Forest Service's Region 6 (Oregon and Washington) program is based at Dorena Tree Improvement Center (Dorena).

The Region 6 program is based upon previous resistance research on western white pine (WWP) and sugar pine (SP) in Idaho and California. Researchers in Idaho identified a suite of eight putative resistance mechanisms in WWP (Hoff and McDonald 1980, Hoff 1988, Hoff and others 2001); these resistances, which became the basis for operational screening at Dorena (Sniezko 1996), are listed in Table 1. Since the mid-1990s, families and individuals within families with a single dominant gene producing a hypersensitive reaction in the needles of SP and WWP have also been identified at Dorena.

Preliminary review of data from the past 15 years of operational screening has contributed to a shift in operational classification of resistance responses at Dorena. Further evaluation will likely lead to further refinements. This paper focuses primarily on rare resistance responses in WWP not previously reported and speculates on the existence of other resistance responses and/or alternative inheritance patterns.

Modifications to Existing Program

Screening for major gene resistance (MGR) was added to the operational program in the mid-1990s. Half-sib families showing a high proportion of stem-symptom-free individuals from operational screening are candidates for MGR screening. Following the protocols of Kinloch and Comstock (1980), seedlings are inoculated at the cotyledon stage and examined for the development of a hypersensitive reaction (HR) in foliar tissue. Frequency of HR in both WWP and SP is very low (Kinloch 1992, Kinloch and others 1999) and varies widely in the natural ranges of these species (Kinloch 1992 and Sniezko and others 2001). Pathotypes of rust virulent to HR in SP and WWP are known (Kinloch and others 1999, Kinloch and Dupper 2002). The pathotype virulent to HR in WWP is present at varying frequencies in many locations in western Oregon (Sniezko and others 2001), while the pathotype virulent to HR in

Richard A. Sniezko, Center Geneticist, and Angelia Kegley, Biological Scientist, for the USDA Forest Service, Dorena Tree Improvement Center, 34963 Shoreview Rd. Cottage Grove, Oregon 97424.

SP is localized to Happy Camp and Mountain Home State Demonstration Forest in California (Kinloch and Dupper 1999).

Several of the resistances identified in the past (Table 1) have been merged into more generic classifications. This does not preclude the existence of these mechanisms but keeps open the interpretation. These broader categories are listed below.

1. No Stem Symptoms—includes needle shed and short shoot phenotypes as well as no spot (Table 1) and seedlings with HR.

2. Delayed Appearance of Stem Symptoms—stem symptoms appear more than one year after inoculation

3. Survival of Trees with Stem Symptoms—amalgamation of the slow canker growth and tolerance categories

4. Delayed Mortality

'New' Resistance Responses in WWP

The four categories listed above involve looking at data from the operational screening program in a slightly different way, focusing on outcomes (e.g. no stem symptoms) rather than attributing the response to a particular resistance mechanism. However, similar phenotypes may be controlled by different genes or have different inheritance; some families exhibit levels of resistance that do not fit the inheritance patterns previously described for some resistance mechanisms. These responses are described below:

Mechanism X—At Dorena in the last decade, it has been noted that half-sib progeny of some WWP phenotypic selections do not fit the single-gene recessive model of needle shed or short shoot mechanisms (Table 1) nor do they exhibit the hypersensitive reaction in the needles of the previously established major gene resistance (unpublished data). Open-pollinated families with this resistance, called Mechanism X, have 40 to 60% seedlings without stem symptoms after artificial inoculation, and early results indicate that these families perform as well as HR families in the field (Sniezko and others in press). This contrasts with Bingham and others (1960); less than 25% of the open-pollinated progeny of first-generation selections were stem-symptom-free. Mechanism X is very low in frequency; progeny of fewer than 30 parent trees are currently known exhibit this resistance. The high frequency of stem-symptom-free individuals in half-sib families suggests that this resistance may be due to a single major gene or relatively few genes. The underlying basis of this response is unknown. However, a form of needle shed has been observed in some of the seedlings from 'X' candidate families (Figure 1, unpublished data).

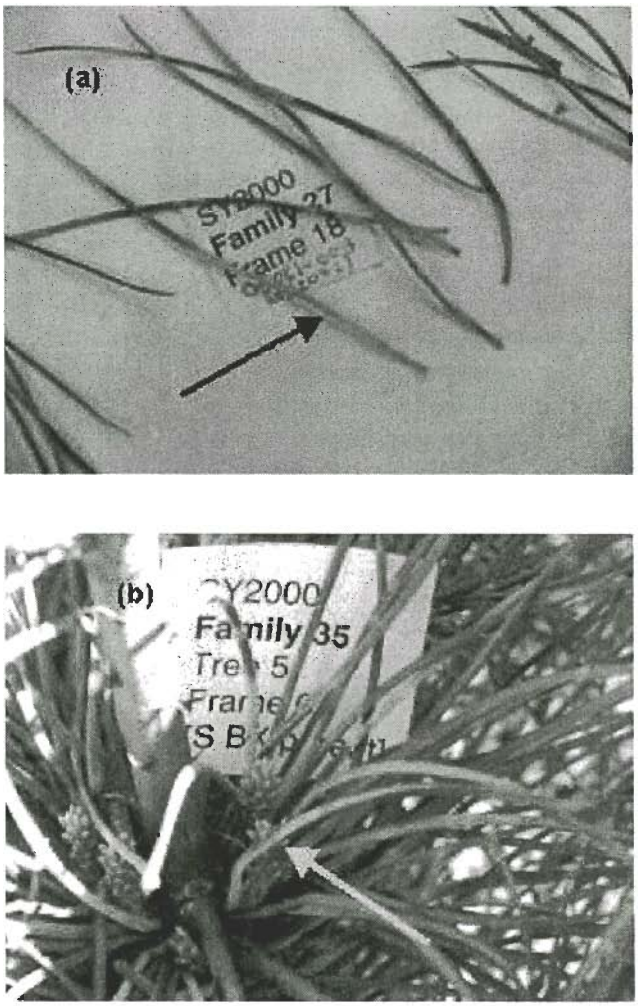


Figure 1 Needle shed in seedlings from the 2000 blister rust screening trial (a) Family 05081-003 (candidate Mechanism X) and (b) Family 21105-052 (Candidate Mechanism Q). Note the lighter (yellow) color at the base of the needles.

Mechanism Q—It has also been noted that some half-sib families with moderate to high proportions of stem-symptom-free individuals also have relatively high levels of bark reaction. These families do not exhibit the hypersensitive reaction in the needles (unpublished data). These families are rare in Region 6 but also occur in families from the Idaho resistance program.

Mechanism Q2—Examination of data from several recent screening trials showed that some families

were not only outstanding for stem-symptom-free and bark reaction, but also the seedlings of those families that did develop stem symptoms (SS) tended to develop SS later, and/or the seedlings tended to live longer relative to other seedlings and families in the same tests. This 'mechanism' may represent a continuum where progress of the fungus is slowed in the needle and/or stem. Thus Mechanism 'Q2' may be a composite of at least four resistance responses and may be related to 'X' and/or 'Q.'

Baseline Resistance Levels

The baseline level of resistance to blister rust is very low in natural populations of WWP and SP in Oregon and Washington; more than 90% of seedlings tested develop stem infections and die (Sniezko and Kegley in press). On average, half-sib progeny of WWP phenotypic selections had less than 3% seedlings without stem symptoms for the 1994 and 1995 screening trials presented here (Table 2).

Performance of Selected WWP Families

Although progeny of most phenotypic selections have very low survival, some families have higher than average levels of several resistance responses (Table 2). The repeatability of these responses has been tested for a number of families, including open-pollinated (OP) families 05081-003 and 21105-052. Families 06024-506 x (504x511) and 06024-506 x (self) are recent crosses among 'resistant' parents. These four families are atypical in their high levels of various resistance responses. All are included in field validation trials.

Family 05081-003 OP is considered a Mechanism X family. First tested in 1987, Family 05081-003 had a high level of stem-symptom-free (56.1%), and 93.8% of the remaining seedlings had bark reactions (unpublished data); those results were validated in the 1995 test. In the 1995 sowing, 56.4% of the seedlings in this family were free of stem symptoms after inoculation (Table 2). Of the remaining seedlings, 60.9% developed bark reactions. A total of 81.8% of the seedlings from this family were either free of stem symptoms or had bark reactions (% Q, Table 2), and sixty-five percent were surviving 5 years after inoculation, including 33% of seedlings with stem symptoms. The percentage Q observed in family 05081-003 suggests the involvement of two major genes, one for stem symptom free and one for bark reaction, but further investigations are needed. In contrast only 6.2% half-sib progeny of 38 newly tested phenotypic selections were either free of stem symptoms or had bark reactions (Table 2), and only 2.5% were surviving five years after inoculation (Sniezko and Kegley in press).

Family 21105-052 OP, a putative Mechanism Q family from a phenotypic selection in eastern Washington, has been used at Dorena since the mid-1990s as a bark reaction checklot. This family has had a consistently high level of bark reaction and a relatively high percentage of seedlings without stem symptoms (Figure 2, Table 2, and Sniezko and Kegley in press). Survival of this family is much higher than for the average half-sib progeny of phenotypic selections; 33-56% of infected seedlings of 21105-052 are alive five years after inoculation (unpublished data).

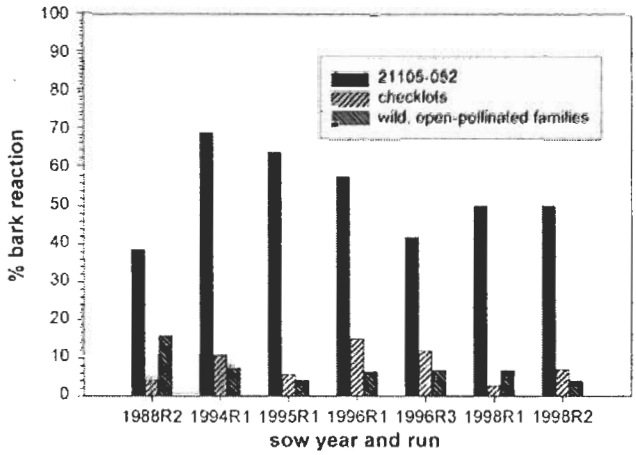


Figure 2: Percent bark reaction of 21105-052 (bark reaction checklot), standard checklots (have HR), and half-sib progeny of phenotypic selections in seven blister rust screening trials at Dorena Tree Improvement Center.

Also summarized in Table 2 from the 1994 test are four full-sib families from the blister rust program in Idaho (Region 1). These families are standard crosses among some of the first confirmed resistant selections and are included in the Idaho blister rust resistance program (Marc Rust, personal communication). Prior analysis by McDonald and Hoff (1971) suggested that parents 17 and 314 were double heterozygotes for two recessive genes for needle-spots-only. At Dorena, they have levels of survival, bark reaction, and stem symptom free similar to the half-sib family 21105-052 included in this test (Figure 3, Table 2). Neither the Idaho full-sib families nor 21105-052 shows diminished survival when inoculated with a strain of rust virulent to HR ('Champion Mine' strain or vcr2) (Figure 3). In contrast, the standard full-sib Dorena HR checklots show a large decrease in survival when inoculated with vcr2 (Figure 3, also Sniezko and Kegley in press). The four Idaho full-sib families

have moderate (34-53%) levels of Q but lower than 21105-052 OP (Table 2). However, the level of stem symptom free, bark reaction, or Q in these full-sib families is lower than for half-sib family 05081-003 and may involve different genes. The Idaho families along with the half-sib families 05081-003 and 21105-052 also show fewer early stem symptoms and a higher percentage of trees alive with stem symptoms (%Q2, Table 2) relative to the average OP progeny of phenotypic selections.

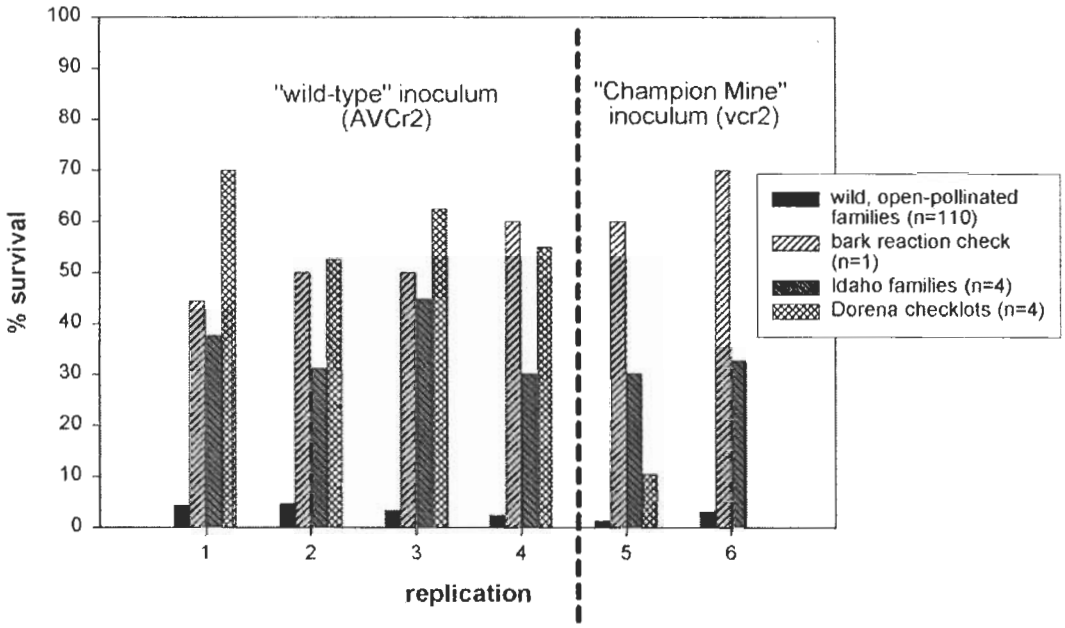


Figure 3: Percent survival of infected seedlings of western white pine (WWP) in a 1994 blister rust screening trial at Dorena Tree Improvement Center, inoculated with rust virulent to HR in WWP ('Champion Mine' strain or vcr2) and avirulent to HR in WWP ('wild type' or AVCr2).

The two full-sib families from 1997 listed in Table 2 were progeny of parents that had a high proportion of bark reaction (unpublished data); they share a common maternal parent. Through the third year after inoculation, more than 50% of the seedlings in those families have not developed stem symptoms, and a moderate to high proportion of the remaining seedlings (those that were not stem-symptom-free) developed bark reactions (Table 2). In contrast 100% of the seedlings of Family 03024-532 OP, included as a susceptible family, developed stem symptoms (Table 2), and only 5 of the original 60 seedlings were surviving three years after inoculation (unpublished data).

Eastern White Pine

The 1995 test at Dorena included four half-sib families of eastern white pine (EWP, *Pinus strobus* L.) from rust-resistant phenotypic selections made many years ago in Wisconsin, Minnesota, and Ontario. Very few EWP were free of stem symptoms (Table 2), and more than 90% developed stem symptoms one year after inoculation (Sniezko and Kegley in press). EWP had more bark reaction than the average WWP (Table 2). However, increased levels of bark reaction did not translate into higher survival; few EWP seedlings survived this test (Sniezko and Kegley in press). While presence of bark reaction is an indication of resistance, there is variation within and among families for size and effectiveness of bark reactions. Slower and less effective production of a wound periderm layer (bark reaction) is associated with a lower level of resistance (Struckmeyer and Riker 1951). Additional breeding may increase the effectiveness of this trait in EWP, WWP, and SP. No comparison is currently available of the resistance of these four families inoculated with rust strains from eastern North America.

Field Validation Trials

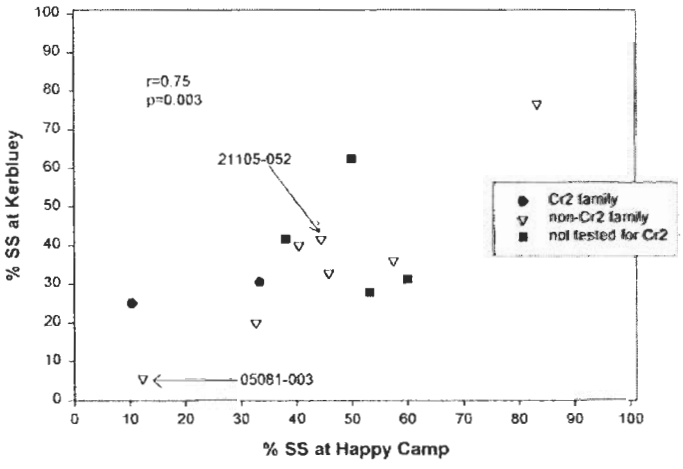
Although operational screening of progeny of phenotypic selections has been ongoing since the 1950s, there have been very few field trials established to validate performance. In the mid-1990s Dorena began establishing a series of replicated field trials. Early results have been reported for the first of these trials, a 1996 planting of 13 WWP and 12 SP at Happy Camp, California. The families were selected to represent a variety of resistance responses on the basis of operational screening results.

Early results for the Happy Camp site indicate that SP is more susceptible than WWP; mean percent stem symptoms was 85.5% for SP and 43.2% for WWP five years after planting (Sniezko and others 2000, Sniezko and others in press). Additionally, the

major gene resistance present in several of these SP families (conferred by Cr1) is ineffective due to the relatively high frequency of a virulent (vcr1) strain of rust at this site. The two WWP families with known major gene resistance (Cr2) were among the families with lowest infection at this site (Sniezko and others in press). The family with the second lowest percentage stem symptoms is family 05081-003, the Mechanism X family described above (Sniezko and others in press). The same 25 families were planted in 1997 in another trial (Kerbluey) on the Umpqua National Forest in the vicinity of Champion Mine, where a strain of rust virulent to Cr2 in WWP exists. There was a strong and significant positive correlation between family mean percent stem symptoms at Happy Camp and Kerbluey for WWP (r=0.75, p=0.003; Figure 4) through 2001. Data from both sites were collected in the fall of 2002 and is in the process of being summarized.

Figure 4 Percent stem symptoms of 13 western white pine families (bark reactions and normal cankers) through 2001 at two field sites.

The results and ideas presented in this paper were generated from the early stages of a summary of



data from many years of blister rust screening at Dorena. The data presented are generally of rare, exceptional families that do not follow previously published inheritance patterns and may represent new unreported resistance responses or lead to new understanding of resistance responses and their inheritance in WWP. Similar observed responses (phenotypes) in different families may be controlled by different genes. For some types of resistance, further breeding will be needed to obtain resistance levels high enough to provide adequate field resistance.

Trait	Phenotype	Hypothesized Inheritance[a]
Reduced needle lesion frequency	Seedling has fewer than average needle lesions	Nondominant gene
No spot formation in secondary needles (no spot)	Seedling exhibits no symptoms of rust (no needle lesions or stem symptoms develop)	?
Early shed of infected secondary needles (needle shed)	Seedling has needle lesions ~9 months after inoculation but no stem symptoms develop	Single recessive gene
Fungicidal reaction in the short shoot (short shoot)	Seedling has needle lesions ~1 year after inoculation but no stem symptoms develop	Single recessive gene
Bark reaction	Seedling exhibits a reaction canker 1, 2, or 3 years after inoculation	
Slow fungus growth	Stem symptoms develops stem symptoms 3 or more years after inoculation	Polygenic
Slow canker growth	Infected seedling has a normal basal canker for 2 or more years and is either alive and stunted or dead 3+ years after developing the canker	Polygenic
Tolerance	Seedling has a normal basal canker for 3 or more years and normal growth 5 years after inoculation	?

Table 1. Resistance mechanisms historically screened for at Dorena Tree Improvement Center

[a]*Adapted from Hoff, R.J. and G.I. McDonald. 1980. Improving rust-resistant strains of inland western white pine. USDA Forest Service Research Paper INT-245. Intermountain Forest and Range Experiment Station, Ogden, UT. 13 p.*

All resistance responses can play a role in restoring WWP, but the durability of each is likely to depend not only on its mode of action but also on the environment. Rust hazard and the evolutionary potential of *C. ribicola* must be considered in deployment strategies. Management strategies for

seedling deployment will have to be refined to help ensure greater durability in blister rust resistance, particularly for race-specific mechanisms, such as HR.

Acknowledgements

The Forest Service, Bureau of Land Management, and other cooperators made the many phenotypic selections and supplied seed for disease testing. Region 1 supplied seed from full-sib checklots. The Region 6 Genetics Program and Forest Health Program have provided funding. Researchers in Idaho, California, and British Columbia provided many insights into the genetics of blister rust resistance. Thanks also to Scott Kolpak for review of an earlier draft of this paper.

References

Bingham, R. T., Squillace, A. E., and Wright, J. W. Breeding blister rust resistant western white pine II. First results of progeny tests including preliminary estimates of heritability and rate of improvement. Silv. Gen. 9(2)33-64.

Hoff, R. J. and McDonald, G. I. 1980. Improving rust-resistant strains of inland western white pine. USDA Forest Service Research Paper INT-245. Intermountain Forest and Range Experiment Station, Ogden, UT. 13 p.

Hoff, R. J. 1988. Blister rust resistance in western white pine for eastern Washington, Idaho, and western Montana. In: Proceedings of a western white pine management symposium. pp. 12-20. Nakusp, British Columbia, May 2-5, 1988.

Hoff, R. J., Ferguson, D. E., McDonald, G. I., and Keane, R. E. 2001. Strategies for managing whitebark pine in the presence of white pine blister rust. In: Whitebark pine communities: ecology and restoration. pp. 346-366.

Kinloch, B. B., Jr. 1992. Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine. Can. J. Bot. 70:1319 1323.

Kinloch, B. B., Jr. and Comstock, M. 1980. Cotyledon test for major gene resistance to white pine blister rust in sugar pine. Canadian Journal of Botany 58: 1912-1914.

Kinloch, B. B., Jr. and Dupper, G. 2002. Genetic specificity in the white pine-blister rust pathosystem, Phytopathology 92(3): 278-280.

Kinloch, B. B., Jr. and Dupper, G. E. 1999. Evidence of cytoplasmic inheritance of virulence in Cronartium ribicola to major gene resistance in sugar pine. Phytopathology 89:192-196.

Kinloch, B. B., Jr., Sniezko, R. A., Barnes, G. D., and Greathouse, T. E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. Phytopathology 89:861-867.

McDonald, G. I. and Hoff, R. J. 1971. Resistance to Cronartium ribicola in Pinus monticola: genetic control of needle-spots-only resistance factors. Can. J. For. Res. 1:197-202

Sniezko, R. A. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon, p. 125-132. In: B.B. Kinloch, M. Marosy, and M.E. Huddleston (eds.). Sugar pine: Status, values, and roles in ecosystems: Proceedings of a symposium presented by the California Sugar Pine Management Committee. Univ. Calif. Div. Agr. Natural Res. Publ. 3362.

Sniezko, R. A., Bower, A., and 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., Kinloch, B. B., Jr., and Dupper, G. E. 2001. Geographic Distribution of 'Champion Mine' Strain of White Pine Blister Rust (Cronartium ribicola) in the Pacific Northwest. Presented at Forest Health Monitoring (FHM) Workshop, Las Vegas, Nevada.

Sniezko, R. A., Bower, A., and Kegley, A. in prep. Variation in Cronartium ribicola field resistance among 13 Pinus monticola and 12 P. lambertiana families: early results from Happy Camp.

Sniezko, R. A. and Kegley, A. J. in prep. Blister rust resistance of five-needle pines in Oregon and Washington.

Struckmeyer, B. E. and Riker, A. J. 1951. Wound-periderm formation in white pine trees resistant to blister rust. Phytopathology 41:276-281.

Table 2. Resistance responses of several families from recent white pine blister rust screening trials at Dorena Tree Improvement Center

Sow Year[a]	Run[b]	Species[c]	Family	Cross Type[d]	height (cm)[e]	NL mean[f]	% infection[g]	% stem symptom free[h]	% bark reaction	% Q[i]	% alive with stem symptoms		% early stem symptoms[j]	%Q2[k]
											3 years	5 years		
1994	1	WWP	17 x 293[l]	CC	56.2	2.5	98.3	13.3	47.1	53.3	47.1	23.1	39.2	71.7
1994	1	WWP	221 x 220[l]	CC	41.7	3.3	100	22.4	17.1	34.5	24.4	15.6	41.5	72.4
1994	1	WWP	208 x 314[l]	CC	45.0	2.0	96.7	25.4	34.1	49.2	53.7	22.7	22.0	89.8
1994	1	WWP	222 x 225[l]	CC	47.2	3.2	100	17.2	25.5	37.9	23.4	18.8	51.1	65.5
1994	1	WWP	21105-052	OP	46.4	2.3	100	28.1	69.2	75.4	76.9	41.5	33.3	89.5
1994	1	WWP	wild families (avg)[m]	OP	42.3	2.6	99.5	2.8	7.2	9.8	4.5	0.7	71.2	35.3
1995	1	WWP	21105-052	OP	38.7	2.8	100	8.6	64.2	67.2	41.5	28.3	60.4	77.6
1995	1	WWP	05081-003	OP	41.8	1.9	96.7	56.4	60.9	81.8	39.1	33.3	13.0	96.4
1995	1	EWP	P-312-5230A	SO OP	40.2	2.1	100	5.7	26.0	30.2	4.0	2.0	92.0	35.8
1995	1	EWP	P-343-5232A	SO OP	47.2	2.0	98.3	3.4	32.1	34.5	5.4	3.6	94.6	36.2
1995	1	EWP	ONT-500-7807	SO OP	52.6	2.2	100	0.0	55.2	54.2	3.4	0.0	100.0	54.2
1995	1	EWP	ONT-4-3465A	SO OP	47.4	3.2	100	0.0	39.2	39.2	0.0	0.0	100.0	39.2
1995	1	WWP	wild families (avg)[m]	OP	35.0	2.6	99.2	2.2	4.1	6.2	1.2	0.8	84.8	20.0
1997	1	WWP	(06024-506) x (504x511)	CC	38.2	1.9	96.6	59.3	72.7	88.9	77.3	--[n]	18.2	98.1[n]
1997	1	WWP	06024-506 x self	CC	33.2	1.7	94.7	52.1	43.5	72.9	47.8	--[n]	34.8	89.6[n]
1997	1	WWP	03024-532	OP	43.7	2.7	100	0.0	0.0	0.0	8.9	--[n]	69.6	33.9[n]

[a] Each trial at Dorena is identified by the year in which it is sown
[b] Run refers to the unit of families inoculated in one test
[c] Where species WWP= western white pine and EWP=eastern white pine
[d] Where Cross Type refers to the family unit: CC=full sib family from a controlled pollination, OP=half sib family, SO OP=half-sib family from a seed orchard
[e] Family mean height approximately three years after sowing
[f] NL mean refers to family mean needle lesion class, a value ranging from 0 to 4 indicating number of needle infections present one year after inoculation with *C. ribicola*
[g] Percentage seedlings with needle lesions and/or stem symptoms
[h] Percentage seedlings that did not develop stem symptoms (cankers or bark reactions)
[i] Q is the percentage seedlings without stem symptoms or having a bark reaction
[j] Percentage seedlings with stem symptoms present one year after inoculation relative to the percentage seedlings with stem symptoms present 3 years after inoculation
[k] Percentage seedlings without stem symptoms plus seedlings with bark reaction, latent stem symptoms and alive with stem symptoms
[l] Full sib checklot from Idaho resistance program
[m] Average of half-sib progeny of 110 phenotypic selections in 1994 test and 38 phenotypic selections in 1995 test
[n] Data for 1997 trial are only through the third year after inoculation