Resistance to White Pine Blister Rust in North American Five-Needle Pines and *Ribes* and Its Implications

Paul J. Zambino and Geral I. McDonald

Abstract—Both R-gene and multigenic resistance to the introduced pathogen white pine blister rust (*Cronartium ribicola*) appear to be present in five-needle pines and *Ribes* of North America. R-gene resistance that confers immunity is well documented in some populations of three North American species of five-needle pines. Rust virulence factors have overcome two of these R-genes in local areas. Partial resistance in pines is due to the interaction of at least several genes and is sensitive to environment. Resistance in wild *Ribes* species may also be due to a combination of R-gene and multigenic resistance. R-gene resistance might be inferred from 1) dominant resistance in related Eurasian *Ribes* and 2) patterns of susceptibility in cross inoculations of *Ribes* clones with rust from different geographic sources. If R-gene resistance occurs in North American *Ribes*, additional undetected blister rust virulence factors may exist in North America. Either a "cost of virulence" or local adaptation might cause reduced rust aggressiveness in local populations, although local adaptation to increased aggressiveness is also conceivable. Reductions in rust aggressiveness might enhance, and increases in aggressiveness might erode the effectiveness of multigenic resistance in pines and *Ribes*. The future effectiveness of both R-gene and multigenic forms of resistance of both hosts may thus be dependant upon the combined effects of resistance traits artificially deployed or generated through natural selection; local or regional blister rust structure with differences in virulence, aggressiveness, and local adaptation; and diversity of environments. **Keywords—**R-gene, partial resistance, virulence, aggressiveness, mortality analysis

Overview

Blister rust was introduced to North America around the turn of the twentieth century. In concert with changes in forest management that it precipitated, it has been and continues to be the most significant factor in the loss of ecosystems in which five-needle pines have been historically important. This heteroecious rust fungus requires two different hosts (five-needle pines and *Ribes*) to complete its life cycle. The two predominant selectable forms of disease resistance found in plants—R-gene and multigenic resistance—have each been identified in both hosts of the white pine blister rust fungus in North America. This paper reviews the general operation of these mechanisms in better known rust pathosystems, their occurrence in five-needle pines and *Ribes*, and the implications of their presence and utilization toward natural or accelerated development of stable blister rust pathosystems that enhance ecological and timber values. Information on five-needle pine breeding programs that contributed most of our knowledge of blister rust resistance has been separately reviewed (McDonald and others, 2004 in press).

Characteristics of R-gene and Multigenic Resistance

R-gene resistance (often referred to as Major Gene Resistance or MGR) results from specific recognition between host and pathogen, with a resistance allele in the host controlling a specific response to pathogenic strains that carry an allele for avirulence at a virulence/avirulence locus (Takken and Joosten 2000). R-gene resistance is usually fully expressed in even juvenile tissue and is effective across different levels of inoculum and host environments. As predicted by gene-for-gene theory (Flor 1956; 1971), virulent[2] races of a pathogen having different or mutant alleles at the avirulence locus overcome R-gene resistance. Thus, R-gene resistance is also known as race-specific resistance, vertical resistance, or gene-for-gene resistance. Resistance in the host and avirulence in the pathogen are both generally considered dominant traits with two readily discerned phenotypes. However, partially dominant expression is known for R-genes in cereal rusts (Roelfs 1988), and unlike the known MGR genes for resistance to blister rust, most R-genes in other rust pathosystems do not confer "all-or-nothing" immunity in resistant interactions, but rather, result in smaller sizes of

In: Geils, B. W. comp. 2004. Proceedings of the 51st Western International Forest Disease Work Conference; 2003 August 18–22; Grants Pass, OR. Flagstaff, AZ: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Paul J. Zambino is Research Plant Pathologist and Geral I. McDonald is Emeritus Research Plant Pathologist at USDA Forest Service, Rocky Mountain Research Station, 1221 S. Main St., Moscow, Idaho 83843 U.S.A.

[2] To avoid confusion, we use the term virulence in this paper in the standard plant pathological context, meaning ability of a strain to avoid detection by a host carrying a specific gene for resistance. We use the term aggressiveness to include quantitative, non-specific traits that affect rates of infection, colonization, host utilization, disease and mortality. In contrast, virulence in the context of human and animal epidemiological literature specifically refers to the ability of a strain of a pathogen to induce mortality.

individual infections than with susceptible interactions (Kolmer 1996; Roelfs 1988). Dominant epistasis is the usual expectation for a resistance phenotype of a host carrying multiple R-genes, so that the amount of rust development is restricted to that allowed by the single most effective gene. Modifier genes in either the host (for example, suppressers of resistance or of hypersensitive cell death) or the pathogen (suppressers of avirulence) can mask recognition, alter the phenotypes of resistance, and change dominant/recessive behavior in crosses (Kolmer 1996; Roelfs 1988, Williams and others 1992; Yu and others 2001; Zambino and others 2000).

In contrast to R-gene resistance, multigenic resistance is quantitative, with complex genetic inheritance. Furthermore, the threshold level of resistance for effective disease control can vary with host physiology, environment, and aggressiveness of pathogenic strains. Multigenic resistance is least effective in young plants and non-hardened tissues, and is thus subject to breakdown under environments or cultural conditions that increase frequency of exposure to a pathogen or that maintain host tissues in a juvenile, succulent state; but relationships are not always predictable (Poteri and others 1997). This, along with the variation and different levels of expression that is usually found among progeny of test crosses under experimental conditions, can make assigning "resistant" and "susceptible" classes among progeny and assigning levels of resistance to parents difficult and subjective.

As shown in the cereal rust systems where it has been most intensively studied, some forms of multigenic resistance that are known as "adult plant resistance" can decrease initial infection, increase latent period between infection and symptom expression, and reduce rates of colonization and sporulation on non-seedling plants (Broers 1997; van der Gaag and Jacobs 1997). However, it must also be recognized that some forms of cereal adult plant resistance and "partial" resistance are not multigenic; but they are instead attributable to R-genes that have defined races of rust that can overcome their partial effectiveness. Furthermore, and contrary to the usual expectations of dominant epistasis, some such R-genes behave similarly to multigenic resistance when combined in a host, by having earlier and more complete expression of recognition, and thus generate more effective "partial" resistance than with single R-genes (Bender and others 2000; Roelfs 1988).

Differences in aggressiveness of pathogenic strains may also affect the landmarks of infection and rust development, thereby altering the effectiveness of multigenic resistance. "Defeated" R-genes can have a residual effect on resistance of hosts that carry them (Nass and others 1981). Avirulence genes serve various necessary functions in fungi, so a cost of virulence may be associated with overcoming R-gene resistance (Frank 2000; Knogge and Marie 1997). Experimental evidence for a cost of virulence has been provided by Thrall and Burdon (2003) and used to explain why strains of flax rust with unnecessary virulence traits are infrequent in host populations with few R-genes, even when in close proximity with host populations that possess higher numbers of R-genes. A similar cost of virulence may be associated with traits that overcome R-gene resistance of blister rust hosts, whether pine or *Ribes*. If this is true, then the geographic distributions of R-genes and multigenic resistance in pine and *Ribes* hosts and of virulence genes in rust are important for understanding the potential for blister rust to become a "naturalized" part of a stable pathosystem that will allow recovery of ecosystems once dominated by five-needle pines.

R-gene Resistance in Five-needle Pines

R-gene resistance to white pine blister rust is known in four species of five-needle pines and is identified by tan-colored needle spots that result from hypersensitive cell death, an indication of host reaction to the fungus. The *Cr1* locus is found in sugar pine (*Pinus lambertiana*) with an average frequency of the resistant allele at about f = 0.02, but ranging from near zero in the northern part sugar pine's range, to about f = 0.07 in some areas in the southern Sierra (Kinloch 1992). *Cr2* is found in western white pine (*P. monticola*) at very low allelic frequencies, predominantly in the western Cascades where it reaches f = 0.001 and the Sierra where it can reach f = 0.004 to 0.008. *Cr2* is as yet undetected among western white pine seedlings representing Rocky Mountain populations (Kinloch and Dupper 2002; Kinloch and others 1999, 2003). *Cr3* in southwestern white pine (*P. strobiformis*) occurs at frequencies of up to f = 0.05; it is unknown whether the same or a different gene is responsible for the even higher frequencies of hypersensitive reactions reported for some stands of the closely related species, limber pine (*P. flexilis*) (Kinloch and Dupper 2002). R-gene resistance has not yet been detected among families of whitebark pine (*P. albicaulis*) tested for resistance to blister rust (Kegley and others this proceedings, Zambino unpublished data).

Mendelian ratios among seedling progeny expressing versus lacking hypersensitive resistant needle spots identified *Cr1* resistance as the first resistance trait in a forest tree species under the control of a single dominant allele. This locus has also been genetically mapped using molecular markers (Devey and others 1995). An outbreak of rust among large numbers of resistant sugar pine carrying the *Cr1* trait at a location near Happy Camp, CA in the Klamath National Forest in northern California revealed that virulence trait *vCr1* (for virulence to *Cr1*) could

become established after periods of intensive regional rust pressure when the "selective" resistant host is present (Kinloch and Comstock 1981). However, spread of *vCr1* has been minimal. Since its detection around two decades ago, strains carrying this virulence have only been found within a few kilometers of two sites—the Happy Camp location and a second site within Mountain Home Demonstration Forest in southern California, over 700 km distant from the original site (Kinloch and Dupper 1987; Kinloch and Dupper 2002).

In contrast to the sugar pine *Cr1* resistance, selection of material carrying what is now known as *Cr2* resistance in western white pine had initially been based on quantitative comparisons of resistance among families, without recognition of the single-gene nature of the resistance. The outbreak near Champion Mine, OR, of a strain that overcome the resistance of many of the trees that had survived earlier epiphytotics (McDonald and others 1984) prompted new tests of seedling progeny of formerly resistant trees that compared rust inoculum containing versus lacking this so-called "Champion Mine Strain". These inoculations revealed the R-gene resistance now identified as *Cr2* (Kinloch and others 1999) and the rust virulence trait that defeats such resistance, now known as *vCr2*. Kinloch and others (1999) have also suggested a modifier or suppressor of *Cr2* resistance, based on lower than expected numbers of resistant progeny in some white pine crosses. In contrast to *vCr1*, rust strains carrying *vCr2* occur at many locations in the Cascades and the Happy Camp, CA location, where western white pine carrying *Cr2* resistance is found either naturally or in plantations (Sniezko 2002; Sniezko and others 2001).

Consistent with gene-for-gene theory (Flor 1956), the *vCr1* and *vCr2* virulence factors are only effective against *Cr1* and *Cr2* genes, respectively. Resistance of R-gene resistant southwestern white pine (*Cr3*) and limber pine has not as yet been overcome at any known location by a matching virulence trait in the rust (Kinloch and Dupper 2002). Lack of rust races that overcome resistance in southwestern white pine and limber pine has thus far prevented the independence of resistance in these two closely related host species from being tested.

Multigenic Resistance in Five-needle Pines

In contrast to the ease with which R-gene resistant and susceptible pine seedlings can be differentiated by hypersensitive-response versus non-reactive needle infections, other forms of resistance to blister rust can vary greatly in expression. Non-R-gene resistance limits the infection rate and subsequent damage, but resistance is not absolute. For this reason, non-R-gene resistance to blister rust is also variously known as "partial resistance" or "slow rusting resistance". The expression of partial resistance in various plant parts appears to be similar for western white pine, sugar pine, and eastern white pine (*P. strobus*) (Bingham 1983; Hoff and McDonald 1972; Hunt 1997; Kinloch and Byler 1981; Kinloch and Davis 1996; Patton 1972; Patton and Riker 1966, Riker and others 1953). Indications of resistance include—1) lack of or low frequency of spot infections on needles; 2) lack of stem infection after needle infection (either through lack of timely colonization through needles and fascicle bases or through premature shed of needles; McDonald and Hoff 1970); 3) inactivation of infections in stems accompanied by formation of a layer of phellogen known as "corking out" (Struckmeyer and Riker 1951) or "bark reaction" (Hoff 1986); and 4) latent infections, slow canker growth, slower mortality of seedlings with needle or stem infection, and "tolerant" responses to infection due to intermittent or decreased overall rates of colonization (Hoff 1984; Hunt 1997; Kegley and Sniezko 2004). Another type of expression termed "twig blight", has been reported in sugar pine (Kinloch and Davis 1986), may have been observed in eastern white pine by Riker and others (1953), and has also been independently observed in eastern white pine (Zambino, unpublished). In this resistance response, a rapid dieback of infected twigs occurs that includes uninfected tissue on either side of the infection, often extending basipetally to the next node.

Resistance may increase with age of seedlings and trees. With this situation, seedlings that would be killed if inoculated at an early age may have increased resistance and longer survival if inoculation is delayed. The same individuals may tolerate infection or overcome infection as older plants in field plantings (Kinloch and Davis 1986; Patton 1961; Riker and others 1953; Zsuffa 1953). Needle type and physiological age (for example, the simple, first formed "primary" needles versus mature "secondary" needles bound in fascicles) can greatly affect infection (Pierson and Buchanan 1938b). Frequency of substomatal vesicles produced by the rust after inoculation is higher and infections and spots more frequent in primary needles and in young expanding secondary needles than in older secondary needles (Patton 1961, 1967). Additionally, some "select" parent trees of sugar pine (Kinloch and Byler 1981; Kinloch and Davis 1986) and eastern white trees (Patton 1961, 1972; Patton and Riker 1966; Zsuffa 1981) that lack or have undetectable levels of resistance among offspring seedlings have a high degree of resistance among grafted scions exposed to the same inoculation conditions. Such mature clonal parents have been characterized as having "mature tree" or "ontogenic" (developmentally expressed) resistance. A portion of this increased resistance may be due to the act of grafting itself (Patton 1961; Patton and Riker 1966). Also, in the opinion of this paper's authors,

this increased resistance could correspond to a slow and progressive increase in the effectiveness of low level resistance, in combination with induced or systemic acquired resistance after exposure to as yet uncharacterized stresses or challenges (for examples see Bonello and others 2001; Enebak and Carey 2000; Evensen and others 2000; Krokene and others 1999, 2000; Pei and others 2003).

Bingham and others (1960, 1969) and Becker and Marsden (1972) have estimated heritability of multigenic resistance of western white pine, based on the occurrence of active cankers on seedlings two years after inoculation. Although differences were apparent in levels of total and additive heritability, these estimates predicted significant progress in obtaining generally effective levels of resistance after several cycles of selection; and this prediction appeared to be borne out by second generation testing (Hoff and others 1973). Partial ("slow-rusting") resistance in sugar pine (Kinloch and Byler 1981) and eastern white pine has also been suggested to be "polygenic" (Heimberger 1972; Zsuffa 1981).

A critical consideration is whether partial resistance phenotypes represent the expression of different mechanisms under different genetic control or multiple modes of expression for one or more common resistance mechanisms, under common genetic control. Though attempts have been made to determine heritability of some modes of resistance expression as individual traits (for example, Hoff 1986; Hoff and McDonald 1971, 1980; McDonald and Hoff 1970; Yanchuk and others 1994), difficulties are posed by the diverse modes of expression and the lack of families that have only one mode of expression. Hoff, in his 1986 analysis of the inheritance of bark reaction as an independent mechanism, acknowledged this problem: "…a high proportion of the seedlings could not be used for determining bark reaction inheritance because many expressed other resistance phenotypes." He further stated that bark reaction was most prevalent among controlled-cross progenies of parents that exhibited intermediate, rather than high expression of this trait. These results may imply that for these data or families, bark reaction may be an intermediate mode of expression of one or more resistance mechanisms. At higher levels of effectiveness (in other words, more contributing alleles or loci), these resistance mechanisms might prevent infection from reaching the stem in the first place, and might therefore be classified as needle shed or "needle spots only" resistance. That genetic mechanisms underlying bark reaction may also be common to other expressions of resistance is consistent with previous observations. Most highly resistant trees, which were derived from parents with superior field resistance and had been selected as highly resistant progeny after seedling screening, produced progeny with highly expressed resistance mechanisms that were "early-operating" and expressed in needles or needle fascicles (Hoff and others 1973).

Multiple genetic mechanisms may underlie heritable differences for at least some partial resistance phenotypes. For example, Woo and others (2001) found that a portion of western white pine families having low needle-spot numbers had an identifiable physical trait—stomatal openings that were narrower than other low-spotting or normal-spotting families. Other mechanical or physiological traits of western white pine (Pierson and Buchanen 1938b) and eastern white pine (Hirt 1938; Patton 1961) change with needle and seedling age and alter frequencies of needle infections, but whether family differences in resistance can reflect differences in the rate of these maturation phenomena has not been explored (Patton 1967). One post-infection, physiological explanation for differences between eastern white pine families in colonization rate, and perhaps also for numbers of "successful" needle and stem colonization events, has been revealed through histological tests of infected needles. These tests revealed greater production of phenolic compounds after infection, less hyphal colonization, and more hyphal disruption in the resistant families that show fewer/smaller spots and less crown damage than more susceptible families with phenolic compounds present at a greater distance from infection in the resistant families (Jurgens and others 2003).

Race-specific interactions are another mechanism proposed by McDonald and Hoff (1975) to account for low spot number for some selections of western white pine from populations from the Interior Northwest. McDonald and Hoff observed that numbers of red-plus-yellow spots that developed on individual, fully susceptible seedlings after experimental inoculations were approximately equal to the added averages from yellow-spot-only and red-spot-only seedlings. They hypothesized that numbers of red versus yellow susceptible spots in seedlings might actually reflect interactions of two R-genes, each effective against one of two putative, locally common strains of rust that cause red or yellow spots in seedlings susceptible to these strains. Unfortunately, lack of pure rust strains for inoculation, lack of western white pine controlled crosses and selfs as test material, and administrative redirection of the research program precluded further investigation of this hypothesis.

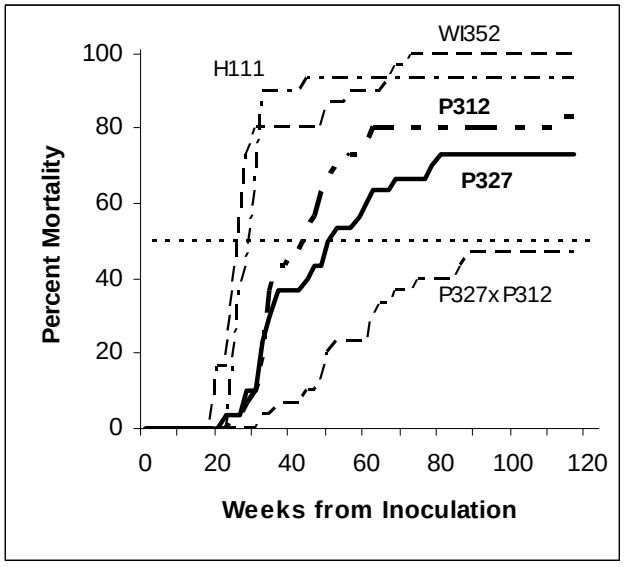
Premature needle-shed resistance may be another resistance phenotype that can result from different genetic mechanisms. This phenotype can occur after hypersensitive response in secondary needles of seedlings that carry R-gene resistance. It has been observed in MGR seedlings of sugar pine and southwestern white pine inoculated with wild type rust, in which case it was often preceded by "bleaching" of infected needles (Kinloch and Zambino,

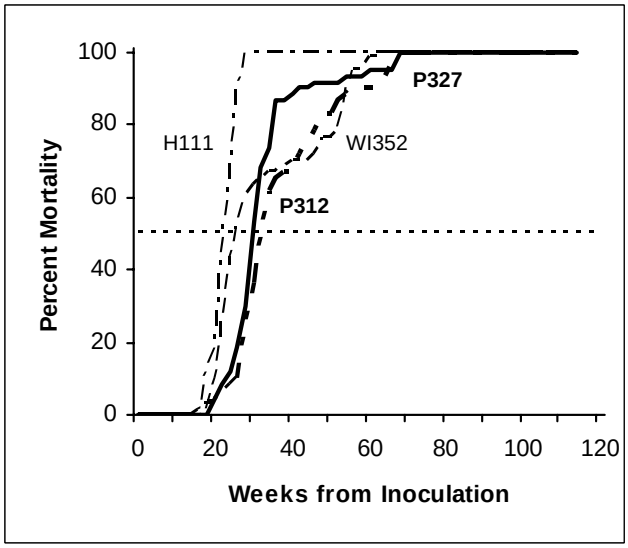
unpublished observations). Needle shed was also reported in western white pine families that can now be assumed to have been carrying *Cr2* (McDonald and others 1984). But, needle shed also occurs in non-MGR coastal western white pine (see discussion of "Mechanism X" and "Mechanism Q", Sniezko and Kegley 2003a,b), in inland western white pine populations where R-gene resistance is not known to occur (McDonald and Hoff 1970), and in eastern white pine seedlings of families known to express high levels of multigenic resistance (Zambino, unpublished observations).

Whether R-gene or multigenic resistance is considered, it is impossible to correctly interpret expressions of resistance without accounting for effects of environment, physiological maturity, and virulence or aggressiveness of local rust variants. This need has resulted in regionally-based programs for testing R-gene and multigenic resistance in different environments against different sources of local rust. This approach is further supported by regional differences in—1) pine populations (Conkle 1996; Rehfeldt and others 1984); 2) distributions of R-gene resistance; 3) expression and effectiveness of partial resistance as affected by environment (examples reviewed separately McDonald and others 2004; Hunt 1997); and 4) occurrence of rust variants that defeat R-gene resistance but uncover forms of resistance normally masked in MGR seedlings (Kinloch and Davis 1996). The British Columbia program considers slow canker growth—a direct measure of effective colonization of host by pathogen—to be the most reliable indicator of broadly effective resistance for their high hazard environment (Hunt 1997, 2002), which may suppress the effectiveness of other resistance mechanisms. For example, resistance of western white pine developed for the Inland Northwest in Idaho that relies heavily on needle mechanisms has held up at relatively dry higher elevation inland sites in British Columbia, but not at coastal sites. It is unknown whether resistance breakdown at coastal sites could be attributable to differences in rust aggressiveness (Meagher 1991) or to environmental effects that alter needle retention in a way that could interfere with some needle-based resistance mechanisms. Hirt's (1939) description allows a comparison of this phenomenon in eastern white pine. An isolated breakdown in resistance of inland-derived materials has also occurred at Merry Creek, ID. At this high-hazard, inland site, the types of undergrowth vegetation on plots with the highest rates of infection may indicate the contribution of abundant soil nitrogen as an environmental aspect of resistance breakdown (McDonald and Decker-Robertson 1998), but the local occurrence of more aggressive strains has also been suggested (McDonald and others 1982). Also, although the bark reaction trait has been effectively used in some selection programs, notably in sugar pine in California (Kinloch and Davis 1996) and in western white pine and sugar pine for the Cascades (Sniezko and Kegley 2003b), the bark reaction symptom on western white pine in British Columbia has been suggested to be due in large part to infection or co-infection with other fungi, and not directly related to blister rust resistance (Hunt 1997).

One potential method for assessing levels of multigenic resistance is to monitor percentages of mortality over time in seedling families that have been inoculated at growth stages that ensure nearly 100 percent early stem infection and maintained under constant environment (Zambino and Michler 1999). Mortality can be considered the outcome of a race between a pathogen's colonization/utilization of host tissue versus host response and growth under that environment. Thus, mortality can be used as a surrogate for canker growth to indirectly assess internal colonization, cellular damage, and degree and timing of physiological resistance response. Research is underway at the University of Minnesota to revive operational resistance testing of eastern white pine (see Patton 1972) using similar mortality-monitoring methods.

Figures 1a,b,c (Zambino, unpublished) demonstrate features of mortality curves among eastern white pine seedlings that were heavily inoculated at two growth stages (Zambino and Michler 1999). These growth stages—uniformly mature, primary needles of 20-week seedlings versus needle expansion stage of secondary needles of 2-0 seedlings—have both been considered highly conducive to needle infection (Hansen and Patton 1977) but would differ in the physiological age of basal stem tissue. Within each experiment, time until mortality of the most susceptible individuals of a family provided an indication of that family's levels of multigenic resistance. Expectations that resistance in open-pollinated families had been inherited as a multigenic trait were reinforced by the even slower onset of mortality in seedlings derived from a cross between resistant parents (figure 1a). Differences among experiments in families' time until onset of mortality and final mortality demonstrate that resistance can be altered by host tissues and host physiology, in contrast with immunity-conferring R-gene resistance. Had R-gene resistance been operating, about half the seedlings from an open-pollinated resistant parent heterozygous at one gene or a fourth of seedlings from a resistant parent heterozygous at two loci would have been fully susceptible, and these ratios would have been constant from experiment to experiment.





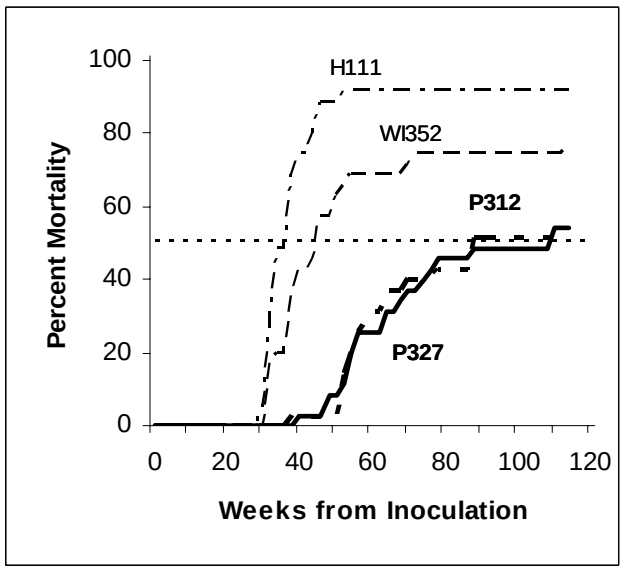


Figure 1—Time until mortality for seedlings of eastern white pine families—a) (top) and b) (middle) subset of families inoculated as 20-week-old seedlings trimmed to 2.0 cm of needle-bearing stem with only primary needles remaining (two experiments, 1 year apart); c) (bottom) subset of families inoculated as 16-month-old, 2-0 seedlings with secondary needles in late expansion. Seedlings were grown in the greenhouse before and after inoculation. All families, including the relatively susceptible H111 and WI352, had been selected as resistant mother-tree candidates from infected stands; mother trees for families P312 and P327 had been selected by Patton. Open-pollinated and controlled-cross (for P327xP312, figure 1a only) seed were provided by the Oconto River Seed Orchard, Nicolet National Forest, USDA Forest Service Region 9 Genetic Program.

Others (Heimberger 1972; Patton 1961) have commented that when very young materials are heavily inoculated, even the most resistant material dies, precluding data analysis. However, using mortality analysis, the consistent gap between the onset of mortality in resistant versus susceptible families in figure 1 still allows identification of resistant families (although differentiation still appears best if older seedlings are used). Also the same single-spore-derived rust strain was used throughout this set of experiments. In the future, different rust strains could be used to examine the effect of aggressiveness in the balance between pathogen colonization and host response; and to test whether part of the apparently multigenic resistance could be due to partially effective R-genes that could be partially overcome by as-yet-undetected virulence factors. Effects of temperature and light regimes could also be tested.

R-gene and Multigenic Resistance in *Ribes*

Compared to five-needle pines, much less is conclusively known concerning mechanisms and frequency of rust resistance in wild North American *Ribes* species. Little incentive has existed for making selfs and crosses of *Ribes* hosts or making controlled inoculations using single-spore-derived rust strains comparable to similar work with pines. This is less true for commercial species, such as red and black currants that are valued as fruit and juice crops, and other ornamental species.

A dominant R-gene (*Cr*) occurs in cultivars Consort, Coronet, Crusader, Titania, Tiben, and Tisel of European black currant (*Ribes nigrum*), where it confers immunity without visible hypersensitive-response lesions. The source of resistance was a homozygous resistant clone of *Ribes pauciflorum* var. *ussuriense* (=*R. ussuriense*; Hunter and Davis 1943; review by Brennan 1996)—an eastern Eurasian species that occurs near the presumed center of diversity for

rusts of five-needle pines (McDonald and others manuscript under review). Immunity conferred by this gene has held up under field conditions in Europe and North America, as well as in recent in vitro tests against single-spore-derived blister rust strains from regions and hosts across North America (Zambino 2000). However, there are also effective levels of multigenic resistance in some varieties of *R. nigrum*, as indicated by their range in rust resistance from extreme susceptibility to moderate resistance (Brennan 1996) and the large amounts of additive vs. non-additive genetic variance for rust resistance in diallel crosses (Zurawicz and others 1996).

The reported complete immunity of red currant Viking, a Norwegian cultivar derived from a cross of *R. petraeum* Wulf. x *R. rubrum* L and also known as Røt Hollandsk Druerips and Holländische Rote (Hahn 1949) may be due to another single dominant gene, in this case from *R. petraeum*. Hahn (1938, 1949) reviewed literature demonstrating the complete lack of infection of this cultivar in extensive field plantings in Scandinavia, Holland, Germany, and Switzerland, and in field trials for susceptibility in Germany (Munich), Great Britain, Canada (Ottawa), and the United States (Maine, New Hampshire, Massachusetts, Connecticut, New York, and Oregon). Because susceptible seedlings have rarely been recovered among even open-pollinated seed derived from this cultivar, Hahn (1938) concluded that the cultivar was homozygous for resistance. Typical symptoms after inoculation in the field (Hahn 1936) or greenhouse (Hahn 1935) are complete immunity, or at most, a watery chlorosis followed by a hypersensitive fleck in very young leaves (Anderson 1939; Hahn 1943). More recently, in vitro and greenhouse inoculation tests by Zambino (2000) of available North American Viking clones produced sporulating infections delimited by slow necrosis at major veins that had been described as typical for red currant cultivars (Hahn 1939; Hennings 1902; Spaulding 1922). Moreover, leaf lobe shape and curvature of this material resembled commercial *R. sativum* red currents but differed from descriptions and photographs from published reports of the hybrid cultivar Viking (Anderson 1939; Hahn 1943). Thus, Zambino (2000) concluded that the test materials might have been misidentified at some time during the six decades of propagation since the last reported resistance tests of this cultivar. Hummer and Picton (2002) have also detected rare infections on artificially inoculated leaves, although infection has not been detected under field conditions at two locations in the western United States (Hummer 2000).

Some cultivars of the North American species *R. aureum*—the "golden" or "Colorado" currant prized as a landscape ornamental—have also been reported to have R-gene resistance (Hunter and Davis 1943). A staminate clone of *R. alpinum* was also reported to be immune (Hahn 1939). In tests that included different rust sources in conjunction with horticultural and wild North American *Ribes*, Anderson and French (1955) also identified a clonal line of *R. hirtellum* that produced large necrotic leaf spots within two weeks of inoculation with some rust isolates, but chlorotic lesions with others, suggesting a differential interaction with rust source. All five California and Oregon rust isolates from sugar pine produced necrotic lesions, whereas five rust isolates from eastern white pine from locations from New York to Minnesota and an isolate from western white pine from Oregon produced chlorotic lesions.

Resistance of Wild North American *Ribes*

Most of the historical record regarding susceptibility of North American *Ribes* that occur in natural habitats has attempted to rank the relative contribution of different species to spread or intensification of the blister rust epidemic on pine. Much less emphasis has been placed on interactions between individual *Ribes* genotypes (bushes and clonally propagated ramets) and individual rust isolates that would be useful for identifying mechanisms of resistance. An early study by Hahn (1928) identified differences in susceptibility among 21 North American *Ribes* species to both the exotic white pine blister rust and the North American pinyon blister rust (*C. occidentale*). Greenhouse-grown whole plants were separately inoculated with these rust species using mixtures of urediniospores representing local collections. *R. triste* was the only species that appeared to be immune to white pine blister rust, whereas other *Ribes* supported rust development to different degrees. Hahn identified some immune plants in some species and differences between host species in their reaction to the two pathogens. However, he suggested that no significant within-species differences were evident among the 2 to 37 plants chosen to represent each *Ribes* species in their reaction to each of the pathogens. Studies by both Kimmey (1938) and Mielke and others (1937) that relied upon artificial inoculations of clones under field conditions also regularly identified immune clones within otherwise susceptible species. The highest proportion of apparently immune clones reported by Mielke and others (1937) was about 15 percent, in a North American black currant, *R. hudsonianum* var. *petiolare*. A small number of additional clones of *R. hudsonianum* var. *petiolare* and other North American species were noted as having high resistance, but lacking immunity (Mielke and others 1937).

It is noteworthy that among western North American species, such apparently high numbers of immune or nearly-immune clones occurred in *R. hudsonianum* var. *petiolare*, although *R. hudsonianum* var. *petiolare* has also shown the greatest potential ability to support urediniospore and teliospore production among western species (Hahn 1928; Kimmey 1938; Mielke and others 1937). The

generally high susceptibility of *R. hudsonianum* var. *petiolare* is understandable, as it is very closely related to the highly susceptible (Kimmey 1938) European black currant, *R. nigrum*. These two species were significant contributors to early spread of blister rust in the West (Lachmund 1934; Mielke and others 1937). These species were also interfertile in controlled crosses (Jandzewski 1907; reviewed in Keep 1962) and were indistinguishable in a study of currant and gooseberry species based on chloroplast DNA restriction sites (Messinger and others 1999). Differences between the most- and least-heavily infected bushes were less dramatic in other species, but relatively resistant bushes with trace infection were also found in *R. viscosissimum* and *R. inerme* (Mielke and others 1937).

The Need to Identify Interactions Among *Ribes* Clones and Rust Sources by Cross Inoculations Under Constant Environment

Both immune and non-immune expressions of resistance to blister rust appear to occur in North American *Ribes*, yet as with pines being examined for multigenic resistance, environment and developmental stage can also greatly affect the expression of resistance. Leaves are immune when immature (Harvey 1972; Spaulding 1922), become highly susceptible after full expansion, and then decrease in receptivity to infection with age (Lachmund 1934; Pierson and Buchanan 1938a; Spaulding 1922). Susceptibility of aging leaves can be extended if shoot growth is interrupted by induced dormancy (Harvey 1972). Previous work has also demonstrated that infection was greater in the less "hardened" plants that grow in full or partial shade than in open-grown plants in full sun (Hahn 1928, Kimmey 1938, Mielke and others 1937). Type of inoculum also has an effect. Significant infection by urediniospores can occur in *Ribes* leaves that would be 1 to 2 weeks too mature for aeciospore-initiated infection (Pierson and Buchanen 1938a). McDonald and Andrews (1981) also noted that disks from 14- to 21-day-old leaves of *R. hudsonianum* var. *petiolare* will develop fewer infections after inoculation with aeciospores than with urediniospores. These results could be due to leaf maturation interfering with infections that colonize leaf tissue slowly. Aeciospores usually divide their stored resources among multiple germ tubes, leaving fewer resources for the successful penetrating germ tube, in contrast with urediniospores which produce a single, stout, and more rapidly growing germ tube. Also, time from inoculation until uredinia appear is usually about 2 to 3 days slower with aeciospore inoculum than with urediniospore inoculum (Zambino, unpublished observation).

The influences of environment and mixed rust strains preclude the use of previously reported symptom severity for differentiating R-gene resistance from multigenic resistance in *Ribes*. Two independent tests are needed to identify types of resistance—1) the reaction of host tissues of different clonally propagated individuals at their peak of susceptibility should be identified in cross-inoculation tests against diverse pure-genotype rust sources under a common environment; and 2) monogenic versus oligo- or multigenic patterns of inheritance should be determined using *Ribes* progeny tests. Three potential types of resistant interactions might be recognized from cross-inoculation tests—1) reversals in relative susceptibility of two clones when exposed to two different strains of rust may indicate R-genes interacting with rust races carrying different virulence/avirulence traits; 2) when resistance increases or decreases due to rust strain occur among clones but the relative ranking of clones is preserved for a quantitative attribute of infection (for example, number of infections, latent period to spore production, or amount of urediniospore or teliospore production), then hosts may differ in background levels of multigenic resistance and may be responding to differences in aggressiveness or overall fitness of rust strains; and 3) instead of race-specific resistance, apparent immunity of some *Ribes* clones to a subset of rust strains could indicate an effectiveness threshold for a quantitative resistance trait interacting with levels of aggressiveness. In this situation, the aggressiveness or colonization ability of a rust strain may be insufficient to overcome the level of partial resistance of the host under the test environment. Diagnosis of this third type of resistant interaction may be aided by determining whether the non-infecting strain has less than average development on other *Ribes*.

Although inheritance of rust resistance is not well studied for wild *Ribes*, the cross-inoculation approach has been employed in attempts to identify/detect differences among *Ribes* clones and rust races. Anderson and French (1955) tested resistance of unreported numbers of clones of several North American *Ribes* species using blister rust collections from different hosts and geographical regions. Although these rust collections cannot be considered as single genotypes, differential interactions reflecting differences in rust sources were reported to occur among some clones of *R. cynosbati* and *R. lacustre*. However, *R. virburnifolium* was uniformly immune, whereas *R. hudsonianum* var. *petiolare, R. cereum*, and *R. americanum* were uniformly susceptible. Of *Ribes* species that displayed variable levels of rust resistance, resistance of even the best clones fell short of complete immunity. Reversals in resistance among some of Anderson and French's (1955) clones when exposed to different rust collections perhaps indicated R-genes; the occasional infections could have been due to R-genes that confer less than full immunity or to a mixture of strains in the inoculum.

All three types of resistant interactions may be represented in cross-inoculation studies using North American *Ribes*. In an attempt to identify *Ribes* host resistance, rust aggressiveness, and environmental factors important for understanding the behavior of rust epidemics, a method was developed by which leaf disks of different clones at physiologically susceptible stages could be uniformly inoculated with rust of different sources and spore types, and held in different environments while tracking development/spore production of uredinia and telia (McDonald and Andrews 1980, 1981, 1982). Rust aeciospores collected from white pine from four locations (Champion Mine, OR; Still Creek, OR; Priest River, ID; and Merry Creek, ID) was used to inoculate 21- to 24–day-old leaf disks of 50 clones representing four *Ribes* species prevalent in the Northwest (*R. hudsonianum, R. inerme, R. lacustre*, and *R. viscosissimum*; McDonald 2000; a discussion of the relative importance of these species in blister rust epidemiology is provided by Kimmey 1938). Infection parameters examined included numbers of infections per unit leaf area, infection efficiency per spore, and time until appearance of uredinial pustules.

Previous analysis of the data either averaged over clones (McDonald 2000) or illustrated strain-specific and location-specific resistant interactions between a variety of geographic sources of rust and clones of *R. hudsonianum* var. *petiolare* from one location (McDonald 1996). Results indicated that 1) the Champion Mine aeciospores ranked at or near the bottom for infection efficiency for *Ribes* species collected from all sites; 2) the Merry Creek inoculum was among the most effective inoculum sources for *Ribes* species and clones from Idaho, but ranked low in effectiveness as an inoculum source for *Ribes* from the Cascades; and 3) some clones of *R. hudsonianum* var. *petiolare* and *R. viscosissimum* were immune to some or all geographic collections of rust (McDonald 2000).

An illustrative subset of the original data is presented in figure 2 in reaction norm format, which displays the "response" of rust source (in terms of infection density and incubation period until urediniospores appear, respectively) under the "environments" posed by clones of two *Ribes* species (*R. hudsonianum* var. *petiolare* from Merry Creek, and *R. viscosissimum* from Priest River). The best case for a source-specific resistant interaction may be found in figure 2a. The Still Creek inoculum had been second only to the Merry Creek inoculum in infection levels on Merry Creek clones H5, H7, and H8 of *R. hudsonianum* var. *petiolare*, but had dramatically lower infection on clone H1. Thus, the stark contrast on clone H1 between the high levels of infection from Merry Creek and Priest River inocula versus the low level of infection from Still Creek and Champion Mine inocula might represent differences in resistance to strains of the rust carrying different avirulence factors. In contrast, the "immunity" of clone H2 against all rust sources provides little information to allow addition interpretation. However, the relative order of infectivity or aggressiveness (Merry Creek > Still Creek > Priest River > Champion Mine) found in clones H5 and H8 had been preserved in the "nearly immune" clone H7. Thus, it cannot be determined whether clone H2 represents a generally effective R-gene, or alternatively, a somewhat higher level of multigenic resistance than was observed in clone H7. The absence of infection of Priest River clone V1 of *Ribes viscosissimum* by selected rust sources may represent a high resistance threshold in this *Ribes* clone (figure 2b). In this example, time until urediniospore appearance of even the most successful rust sources was relatively slow, suggesting that Champion Mine and Priest River inoculum sources may have lacked the aggressiveness to colonize/develop before leaf disks reached maturation levels that prevented rust development.

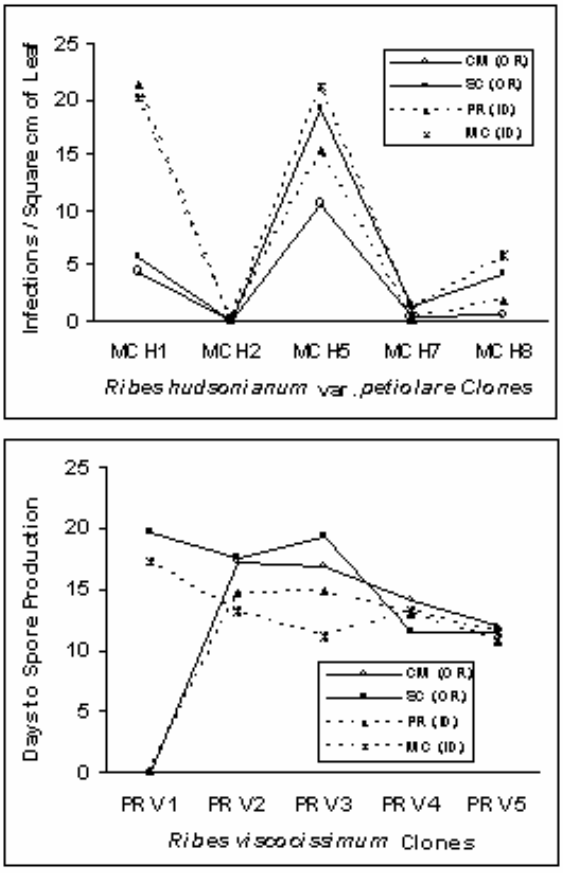


Figure 2—Examples of three types of interactions among *Ribes* clones in cross inoculation studies with rust aeciospores from Champion Mine, OR (CM), Still Creek, OR (SC), Priest River, ID (PR), and Merry Creek, ID. a) (top) Infections per square cm that developed on inoculated, detached leaf disks (McDonald and Andrews 1981, 1982, 1982) of *Ribes hudsonianum* var. *petiolare* clones originating from Merry Creek, ID (McDonald, 1996). b)

(bottom) Days until urediniospores were produced on inoculated, detached leaf disks of clones of *Ribes viscosissimum* originating from Priest River, ID. (Lack of infection is indicated by data points at "0 days"; McDonald, unpublished)

Implications of Aggressiveness on Effectiveness of Multigenic Pine Resistance

As well as identifying differences in resistance among *Ribes* clones, results of Anderson and French (1955), McDonald and Andrews (1982), and McDonald (1996) imply regional differences in rust aggressiveness or virulence to *Ribes*. To assess how these differences may influence the stability and usefulness of multigenic resistance in pine, one needs to determine—1) how the tentatively identified differences in aggressiveness on *Ribes* relate to differences in aggressiveness on pine, and vise versa; 2) whether differences in aggressiveness are due to costs of virulence factors that overcome R-genes in pine or *Ribes*; 3) whether changes in aggressiveness are related to local adaptation; 4) whether the potential for future changes in the rust pathogen will generally favor increased or decreased aggressiveness; and 5) the magnitude of differences in pine infection and mortality that might be attributed to differences in rust aggressiveness.

Little experimental evidence has been obtained regarding the interaction of host resistance in five-needle pine and *Ribes* with rust aggressiveness. The Champion Mine inoculum was of low aggressiveness on *Ribes* as determined by McDonald and others (1982). Subsequently, McDonald and others (1984) reported similar inoculum to have a longer incubation period on susceptible pine. These results suggest that lower aggressiveness on one host may correspond to some aspects of lower aggressiveness on another. Because the 1984 study was completed before the identification of the *vCr2* virulence factor and *Cr2*-resistance-carrying western white pine families, inoculum for neither study had been specifically tested for *vCr2*, although aeciospores had been obtained from trees that may be presumed to carry *Cr2*. Kinloch and others (1999) had compared Happy Camp inoculum (which can be presumed to have a high frequency of *vCr1* in addition to *vCr2*) to other sources of *vCr2* for their ability to infect families of western white pine seedlings carrying *Cr2*. Their finding that Happy Camp inoculum produced the fewest spots and fewest infected seedlings of any inoculum that carried *vCr2* was attributed to the low *vCr2* frequency at Happy Camp at the time of sampling. Time to appearance of symptoms was not reported. *Cr2* western white pine and *Cr1* sugar pine are the only pine hosts at the Happy Camp inoculum source location and occur as separate blocks in close proximity, although *Cr1* sugar pine is by far the predominant pine host represented. The low *Cr2* frequency despite the close proximity of *Cr1* and *Cr2* hosts may be indirect evidence of selection against strains carrying *vCr1* and *vCr2* in combination. As previously mentioned, *vCr1* is of limited geographic distribution (Kinloch and Dupper 1987; Kinloch and Dupper 2002). This limited distribution has been attributed to white pine blister rust in western North America being a genetically fragmented meta-population (Kinloch and others 1998); however, costs of virulence on environmental fitness or aggressiveness on one or both hosts should also be considered. Although Zambino (2002) was able to infect *R. nigrum* cultivars that lacked the *Ribes CR*-gene with strains derived from aeciospores produced on *Cr*-gene carrying sugar pine and western white pine from Happy Camp and Champion mine, respectively, infections with such strains frequently appeared to develop slower and produce fewer uredinia compared to other North American sources of blister rust inoculum (Zambino, unpublished). To date, no tests have compared aspects of infection and colonization of single-genotype rust strains on pine versus *Ribes* hosts; however, such tests are being developed (Zambino, unpublished).

Models and observations based on other host–parasite interactions may provide insights into changes that might occur in white pine blister rust aggressiveness at some localities. Zimmer (2003) stated that according to observations on animal and human pathogens, "…a pathogen can evolve to become harmless, more deadly, or anything in between, depending on the forces guiding natural selection." Examples of evolution from high to lower aggressiveness are believed to occur most frequently when a pathogen is not in equilibrium with its host, such as when an imported pathogen encounters a new, highly susceptible population. To understand this trend, one must consider a pathogen's evolutionary tradeoffs between "…how fast a pathogen breeds and how easily it can infect new hosts" (Zimmer 2003) and the tradeoff between longevity and fecundity (Frank 1996). Interacting factors include—1) the population structure of both host and parasite in relation to their capacity for long or short range dissemination (Haraguchi and Sasaki 2000); 2) the association between aggressiveness and transmission success (Levin 1996); 3) the susceptibility of the host population and its density (Day 2003; Levin 1996); 4) whether infection of an individual characteristically occurs by a single infection with a single strain, by co-infection by several strains, or by super-infection in which a more aggressive strain can replace or eliminate a less aggressive strain on a host (Mosquera and Adler 1998); and 5) whether infections are lethal or sublethal (Schjorring and Koella 2002). Our interpretation of models of aggressiveness evolution holds that in general, high virulence will be favored when a highly susceptible population is being colonized by an efficiently disseminated pathogen; when aggressiveness translates into abundant short-term fecundity (given that abundant susceptible hosts are

present); and when infection is either by single infection or by super-infection that results in rapid mortality. Lower aggressiveness can evolve when host density is low and/or intermittent and infection uncertain, so that pathogen longevity is favored over fecundity; where population structure exists in association with more localized dissemination of host and pathogen; where either co-infection or induced resistance to subsequent infection can occur; and on hosts where infections can be sublethal allowing greater longevity of individual infections.

Characteristics of the blister rust pathogen and five-needle pines during the blister rust epidemic in North America suggest that high pathogen aggressiveness and fecundity would have been favored early in the epidemic. However, rust aggressiveness/fecundity would be expected to decrease over time, eventually reaching levels that are selectively responsive to local host population structure, host density, levels and types of resistance, and environmental signals. Blister rust that reached North America from Europe was probably of high aggressiveness, having spread and intensified rapidly in Europe on eastern white pine in nurseries and small, scattered plantations and on the even more widely planted European black currant (Moir 1924). Aggressiveness and high fecundity would have been initially favored upon initial introduction to the extensive stands of susceptible pines in North America. Mortality was high—over 95 percent in some stands of eastern and western white pine—so that uncolonized hosts would eventually have become scarce. Intermittent regeneration of new five-needle pine cohorts from survivors might have favored those slow-growing cankers that had persisted longer due to higher host resistance/lower pathogen aggressiveness, compared to cankers caused by strains with high fecundity coupled with aggressiveness/lethality to hosts. This process would be more pronounced if hosts infected with less aggressive strains could develop induced or systemic acquired resistance that would limit subsequent infection by aggressive rust strains that cause rapid host mortality.

Is induced resistance to blister rust a feature of North American five-needle pines? Infection records were recently re-examined from diverse stands that developed in northern Idaho within a generation after the arrival of blister rust. This analysis revealed that a significant proportion of the infected trees developed only one or a few cankers (McDonald and others in press). Induced resistance may also operate in very young seedlings. Kinloch and Comstock's (1981) original report of the *vCr1* virulent race described an experiment where sugar pine seedlings carrying *Cr1* were inoculated with wild-type rust (lacking *vCr1* virulence) and subsequently developed hypersensitive resistant spots. These inoculated plants were held under 24-hr photoperiods until secondary needles developed, and were then inoculated a second time, with rust carrying *vCr1*. Despite the normal increase in susceptibility that continuous illumination usually causes in sugar pine seedlings (Kinloch 1980), these previously exposed seedlings developed fewer susceptible needle spots than did susceptible seedlings that had not been previously exposed. This response may represent an example of induced or systemic acquired resistance to white pine blister rust, thereby supporting the premise that rust strains with low aggressiveness could become established and persist in forest trees.

If reduction in rust aggressiveness does occur, what would be the effect on pine resistance developed under either natural or artificial selection? From the outbreaks that occurred in established stands of *Cr1* sugar pine and *Cr2* western white pine, it appears that costs of virulence are not of sufficient magnitude to prevent trees with MGR as a sole source of resistance from rapidly succumbing when challenged with *vCr1*- or *vCr2*-carrying strains, respectively (Kinloch and Comstock 1981; Kinloch and Dupper 1987; Kinloch and others 1999; McDonald and others 1984). However, one would expect that lower aggressiveness would affect the balance between tissue colonization and non-specific host response, and thereby enhance the effectiveness of partial resistance. Even small changes in effectiveness of partial resistance may be critical for pines with marginal resistance, when grown under moderate rust hazard. In fact, we may be reaping the benefits of reduced aggressiveness already—the breakdown of *Cr1* resistance in sugar pine at the Happy Camp location has enabled seedlings carrying *Cr1* to be tested for additional resistant traits that may be oligo- or multigenic (Kinloch and Davis 1996) and similar programs are in place for testing the partial resistance of western white pine carrying *Cr2* by exposing them to rust strains carrying *vCr2* during screening or under field conditions (Sniezko and Kegley 2003b, Sniezko and others 2004). It remains undetermined whether the apparent levels of multigenic resistance among seedlings selected for their *Cr* resistance have been enhanced by lower aggressiveness of *vCr1* and *vCr2*-carrying strains.

It is important to note that one must never assume that lower aggressiveness will be the rule, disregarding the specificity that rust may develop during adaptation to locality. Aggressiveness may even be indirectly affected as rust adapts its life cycle to optimize its infection of pine and *Ribes* hosts under specific climates and microclimates. McDonald (1996) has suggested that rust infection of pine in some areas of the southern Sierra may be more likely to occur during spring weather patterns than in fall. This adjustment would necessitate early production of telia, which has been considered a hallmark of host stress, low pathogen aggressiveness, and/or moderate plant resistance

in many rust pathosystems (Waters 1928). Local fluctuations in *Ribes* and pine hosts following thinning, burning, regeneration, restoration efforts, and fluctuation in types and levels of resistance might also ensure that aggressiveness will continue to fluctuate, depending on the local situation. For restoration, use of R-gene plus multigenic resistance may be favored in those areas where both occur. In theoretical models, greater non-specific resistance stabilizes the gene frequency dynamics of specific defenses (Frank 2000). However, where remnant stands occur, one may expect that local adaptation to local rust and the occurrence of local population structure would support utilizing these remaining trees for regeneration whenever possible. Thus, utilization, development, and deployment of pine resistance will remain an activity that will be aided by knowledge of types and effectiveness of resistance in pine populations, for management by regeneration and restoration efforts at the local level.

References

Ahlgren, C. E. 1955. Grafted selections of eastern white pine tested for resistance to blister rust. J. Forestry 53: 727–729.

Anderson, O. C. 1939. A cytological study of resistance of Viking currant to infection by *Cronartium ribicola*. Phytopathology 29: 26–40.

Anderson, R. L.; French, D. W. 1955. Evidence of races of *Cronartium ribicola* on *Ribes*. Forest Science 1: 38–39.

Becker, W. A.; Marsden, M. A. 1972. Estimation of heritability and selection gain for blister rust resistance in western white pine. In: Biology of Rust Resistance in Forest Trees: NATO-IUFRO Advanced Study Institute: proceedings; 1969 August 17-24; Moscow, ID: USDA Misc. Pub No. 1221: 397–409.

Bender, C. M.; Pretorius, Z. A.; Kloppers, F. J.; Spies, J. J. 2000. Histopathology of leaf rust infection and development in wheat genotypes containing Lr12 and Lr13. J. Phytopathol. 148: 65–76.

Bingham, R. T. 1983. Blister rust resistant western white pine for the Inland Empire: The story of the first 25 years of the research development program. Ogden, Utah: USDA Forest Service Intermountain Forest and Range Experiment Station General Technical Report INT-146: 45 p.

Bingham, R. T.; Olson, R. J.; Becker, W. A.; Marsden, M. A. 1969. Breeding blister rust resistant white pine. V. Estimates of heritability, combining ability, and genetic advance based on tester matings. Silvae Genet. 18: 23–38.

Bingham, R. T.; Squillace, A. E.; Wright, J. W. 1960. Breeding blister rust resistant western white pine. II. First results of progeny tests including preliminary estimates of heritability and rate of improvement. Silvae Genet. 9: 33–41.

Bonello, P.; Gordon, T. R.; Storer, A. J. 2001. Systemic induced resistance in Monterey pine. Forest Pathol. 31: 99–106.

Brennan, R. M. 1996. Currants and Gooseberries. Chapter 3. In: Janick, J; Moore, J. N., eds. Fruit Breeding, Vol. II Small Fruits and Vine Crops. John Wiley & Sons. Inc. N.Y.: 191–295.

Broers, L. M. H. 1997. Components of quantitative resistance to yellow rust in ten spring bread wheat cultivars and their relations with field assessments. Euphytica 96: 215–223.

Conkle, M. T. 1996. Patterns of variation in isozymes of sugar pine. In: Kinloch, B. B. Jr.; Marosy, M.; Huddleston, M., eds. Sugar pine: Status, Values, and Roles in Ecosystems. Symposium of the California Sugar Pine Management Committee: proceedings; 1992 March 30-April 1; Davis, CA. Oakland, CA: Univ. Calif. Div. Agric. Nat. Resour., Publication 3362: 99.

Day, T. 2003. Virulence evolution and the timing of disease life-history events. Trends in Ecol. Evol. 18: 113–118.

Devey, M. E.; Delfino-Mix, A.; Kinloch, B. B., Jr.; Neale, D. B. 1995. Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white pine blister rust in sugar pine. Proc. Natl. Acad. Sci. USA 92: 2066–2070.

Enebak, S.A.; Carey, W.A. 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. Plant Dis. 84: 306–308.

Evensen, P.C.; Solheim, H.; Hoiland, K.; Stenersen, J. 2000. Induced resistance of Norway spruce, variation of phenolic compounds and their effects on fungal pathogens. For. Path. 30: 97–108.

Flor, H. H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9: 275–296.

Flor, H. H. 1956. The complementary genic system in flax and flax rust. Adv. Genet. 8: 29–54.

Frank, S. A. 1996. Models of parasite virulence. Q. Rev. Biol. 71: 37–78.

Frank, S. A. 2000. Specific and non-specific defense against parasitic attack. J. Theor. Biol. 202: 283–304.

Janczewski, E. De. 1907. Monograph of the currants *Ribes* L. Mem. Soc. Phys. Et Hist. Nat. de Genéve 35: 199–517.

Jurgens, J. A.; Blanchette, R. A.; Zambino, P. J.; David, A. 2003. Histology of white pine blister rust in needles of resistant and susceptible eastern white pine. Plant Dis. 87: 1026–1030.

Hahn, G. G. 1928. The inoculation of pacific northwestern *Ribes* with *Cronartium ribicola* and *C. occidentale*. J. Agric. Res. 37: 663–683.

Hahn, G. G. 1935. Immunity of Viking a Norwegian red currant to *Cronartium ribicola* and *C. occidentale* under greenhouse conditions. U.S.D.A. Circ. 330. 16 p.

Hahn, G. G. 1936. Immunity of Viking red currant from white pine blister rust under field conditions. Phytopathology 26: 860–875.

Hahn, G. G. 1938. Blister rust susceptibility studies of naturally pollinated seedlings of the immune Viking currant. J. Forestry 36: 737–747.

Hahn, G. G. 1939. Immunity of a staminate clone of *Ribes alpinum* from *Cronartium ribicola*. Phytopathology 29: 981–986.

Hahn, G. G. 1943. Blister rust relations of cultivated species of red currants. Phytopathology 33: 341–353

Hahn, G. G. 1949. Evidence of the non-existence of physiological races in *Cronartium ribicola.* Phytopathology 39: 85–87.

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

Haraguchi, Y.; Sasaki, A. 2000. The evolution of parasite virulence and transmission rate in a spatially structured population. J. Theor. Biol. 203: 85–96.

Harvey, A. E. 1972. Influence of host dormancy and temperature on teliospore induction by *Cronartium ribicola.* For. Sci. 18: 321–323.

Heimberger, C. 1972. Relative blister rust resistance of native and introduced white pines in eastern North America. In: Biology of Rust Resistance in Forest Trees: Proc. of a NATO-IUFRO Advanced Study Institute: 1969 August 17–24; Moscow, ID. U.S.D.A. Misc. Publ. 1221: 257–269

Hennings, P. 1902. Beobachtungen über das verschiedene Auftreten von *Cronartium ribicola* Dietr. Auf verschiedenen *Ribes*-Arten. Ztschr. Pflanzendr. 12: 129–132.

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

Hirt, R. R. 1944. Distribution of blister-rust cankers on eastern white pine according to age of needle-bearing wood at time of infection. J. For. 42: 9–14.

Hoff, R. J. 1984. Resistance to *Cronartium ribicola* in *Pinus monticola*: higher survival of infected trees. USDA For. Serv. Res. Note INT-343. 6 p.

Hoff, R. J. 1986. Inheritance of the bark reaction resistance mechanism in *Pinus monticola* infected by *Cronartium ribicola*. USDA For. Serv. Res. Note INT-361. 8 p.

Hoff, R. J.; McDonald, G. I. 1971. Resistance of *Pinus monticola* to *Cronartium ribicola*: Short shoot fungicidal reaction. Can. J. Bot. 49: 1235–1239.

Hoff, R. J.; McDonald, G. I. 1972. Stem rusts of conifers and the balance of nature. In: Bingham, R. T.; Hoff, R. J.; McDonald, G. I., eds. Biology of rust resistance in forest trees. Washington, DC: U.S. Dept. Agric. Publ. 1221: 525–535.

Hoff, R. J.; 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.; Bingham, R. T. 1973. Resistance to *Cronartium ribicola* in *Pinus monticola*: Structure and gain of resistance in the second generation. U.S. For. Serv. Res. Pap. INT-245. 13 p.

Hummer, K. E. 2000. 'Viking' red currant. J. Am. Pomological Soc. 54: 54-56.

Hummer, K. E.; Picton D. D. 2002. Pine blister rust resistance screening in *Ribes* germplasm. In: Williamson, B. convener. 8th International Symposium on *Rubus* and *Ribes*: proceedings; 2001 July; Dundee, Scotland. ISHS Fruit Section, International Working Group on *Rubus* and *Ribes.* Acta Hort. 585: 287-291.

Hunt, R. S. 1997. Relative value of slow-canker growth and bark reaction as resistance responses to white pine blister rust. Can. J. Plant Pathol. 19: 352–357.

Hunt, R. S. 2002. Relationship between early family-selection traits and natural blister rust cankering in western white pine families. Can. J. Plant Pathol. 24: 200-204.

Hunter, A. W. S.; Davis, M. B. 1943. Breeding rust resistant black currants. Am. Soc. Hort. Sci. Proc. 42: 467–468.

Keep, E. 1962. Interspecific hybridization in *Ribes*. Genetica 33: 1–23.

Kegley, A.; Sniezko, R. A. 2004. Variation in blister rust resistance among 226 *Pinus monticola* and 217 *P. lambertiana* seedling families in the Pacific Northwest. In: Sniezko, R.; Samman, S.; Schlarbaum, S.; Kriebel, H., eds. Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance. IUFRO Working Party 2.02.15.: proceedings; 2001 July 24-25, Medford, OR, USA. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station RMRS-P-32: 209–226.

Kimmey, J. W. 1938. Susceptibility of *Ribes* to *Cronartium ribicola* in the West. J. Forestry 36: 312–320.

Kinloch, B. B. 1980. Effect of photoperiod and container size on sugar pine seedling growth and infection by white pine blister rust. USDA For. Serv. Res. Note PSW-343.

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.; Byler, J. W. 1981. Relative effectiveness and stability of different resistance mechanisms to white pine blister rust in sugar pine. Phytopathology 71: 386–391.

Kinloch, B. B., Jr.; Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. Plant Dis. 65: 604–605.

Kinloch, B.B., Jr.; Davis, D. 1996. Mechanisms and inheritance of blister rust resistance in sugar pine. In: Kinloch, B. B. Jr.; Marosy, M.; Huddleston, M., eds. Sugar pine: Status, Values, and Roles in Ecosystems. Symposium of the California Sugar Pine Management Committee: proceedings; 1992 March 30-April 1; Davis, CA. Oakland, CA: Univ. Calif. Div. Agric. Nat. Resour., Publication 3362: 125–132.

Kinloch, B. B., Jr.; Dupper, G. E. 1987. Restricted distribution of a virulent race of the white pine blister rust pathogen in the western United States. Can. J. For. Res. 17: 448–451.

Kinloch, B. B., Jr.; Dupper, G. E. 2002. Genetic specificity in the white pine blister rust-blister rust pathosystem. Phytopathology 92: 278–280.

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. Phytopathology 89: 861–867.

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. Phytopathology 93: 691–694.

Kinloch, B. B., Jr.; Westfall, R. D.; White, E. E.; Gitzendenner, M. A.; Dupper, G. E.; Foord, B. M.; Hodgskiss, P. D. 1998. Genetics of *Cronartium ribicola*. IV. Population structure in western North America. Can. J. Bot. 76: 91–98.

Knogge, W.; Marie, C. 1997. Molecular Characterization of Fungal Avirulence. In: Crute, I. R.; Holub, E. B.; Burdon, J. J. eds. The Gene-for-Gene Relationship in Plant-Parasite Interactions. Wallingford, UK: CAB International: 329–346.

Kolmer, J. 1996. Genetics of resistance to wheat leaf rust. Ann. Rev. Phytopathol. 34: 435–455.

Krokene, P.; Christiansen, E.; Solheim, H.; Frenceschi, V. R.; Berryman, A. A. 1999. Induced resistance to pathogenic fungi in Norway spruce. Plant Physiol. 121: 565–569.

Krokene, P.; Solheim, H; Langstrom, B. 2000. Fungal infection and mechanical wounding induce disease resistance in Scots pine. Eur. J. Pl. Pathol. 106: 537–541.

Lachmund, H. G. 1934. Seasonal development of *Ribes* in relation to the spread of *Cronartium ribicola* in the Pacific Northwest. J. Agric. Res. 49: 93–114.

Levin, B. R. 1996. The evolution and maintenance of virulence in microparasites. Emerging Inf. Dis. 2: 93–102.

McDonald, G. I. 1996. Ecotypes of blister rust and management of sugar pine in California. In: Kinloch, B. B. Jr.; Marosy, M.; Huddleston, M., eds. Sugar pine: Status, Values, and Roles in Ecosystems. Symposium of the California Sugar Pine Management Committee: proceedings; 1992 March 30-April 1; Davis, CA. Oakland, CA: Univ. Calif. Div. Agric. Nat. Resour., Publication 3362: 137–147.

McDonald, G. I. 2000. Geographic variation of white pine blister rust aeciospore infection efficiency and incubation period. HortTechnology. 10: 533–536.

McDonald, G. I.; Andrews, D. S. 1980. Influence of temperature and spore stage on production of teliospores by single aeciospore lines of *Cronartium ribicola*. USDA For. Serv. Res. Paper INT-256. 9 p.

McDonald, G. I.; Andrews, D. S. 1981. Genetic interaction of *Cronartium ribicola* and *Ribes hudsonianum* var. *petiolare*. Forest Science. 27: 758–763.

McDonald, G. I.; Andrews, D. S. 1982. Genetic variation of epidemiological fitness traits among single-aeciospore cultures of *Cronartium ribicola*. Phytopathology 72: 1391–1396.

McDonald, G. I.; Decker-Robertson, D. L. 1998. Long-term differential expression of blister rust resistance in western white pine. In: First IUFRO Rusts of Forest Trees Working Party Conference: proceedings; 1998 August 2-7; Saariselkä, Finland. Finnish Forest Research Inst., Research Papers 712: 285–295.

McDonald, G. I.; Hansen, E. M.; Osterhaus, C. A.; Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Dis. 68: 800–804.

McDonald, G. I.; Hoff, R. J. 1970. Resistance to *Cronartium ribicola* in *Pinus monticola*: early shedding of infected needles. USDA For. Serv. Res. Note INT-124. p.

McDonald, G. I.; Hoff, R. J. 1975. Resistance to *Cronartium ribicola* in *Pinus monticola*: an analysis of needle-spot types and frequencies. Can. J. Bot. 53: 2497–2505.

McDonald, G.; Zambino, P.; Sniezko, R. 2004. Breeding rust-resistant five-needle pines in the western United States: Lessons from the past and a look to the future. In: Sniezko, R; Samman, S.; Schlarbaum, S.; Kriebel, H., eds. Breeding and genetic resources of five-needle pines: growth adaptability, and pest resistance. IUFRO Working Party 2.02.15: proceedings; 2001 July 24-25; Medford, OR, USA. Fort Collins, CO: U.S.D.A. Forest Service, Rocky Mountain Research Station. RMRS-P-32.

Meagher, M. D. 1991. A joint U.S.–Canada blister rust "races" test on *Pinus monticola*: First-year results. In: Hiratsuka, Y.; Samoil, J. K.; Blenis, P. V.; Crane, P. E.; Laishley, B. L., eds. Rusts of Pine. 3rd IUFRO Rusts of Pine Working Party Conference: proceedings; 1989 September 18–22; Banff, Alberta, Canada. Edmonton, Alberta: For. Can., Northwest Region, North. For. Cent., Inf. Rep. NOR-X-317: 206–218.

Messinger, W., K. Hummer, and A. Liston. 1999. *Ribes* (Grossulariaceae) phylogeny as indicated by restriction-site polymorphisms of PCR-amplified chloroplast DNA. Plant Systematics and Evolution 217: 185–195.

Mielke, J. L.; Childs, T. W.; Lachmund, H. G. 1937. Susceptibility to *Cronartium ribicola* of the four principal *Ribes* species found within the commercial range of *Pinus monticola*. J. Agric. Res. 55: 317–346.

Moir, W. S. 1924. White pine blister rust in western Europe. U.S.D.A. Bull. No.1186. 32 p.

Mosquera, J.; Adler, F. R. 1998. Evolution of virulence: a unified framework for coinfection and superinfection. J. Theor. Biol. 195: 293–313.

Nass, H. A.; Pederson, W. L.; MacKenzie, D. R.; Nelson, R. R. 1981. The residual effect of some "defeated" powdery mildew resistance genes in isolines of Chancellor winter wheat. Phytopathology 71: 1315–1318.

Patton, R. F. 1961. The effect of age upon susceptibility of eastern white pine to infection by *Cronartium ribicola*. Phytopathology 51: 429–434.

Patton, R. F.; Riker, A. J. 1966. Lessons from nursery and field-testing of eastern white pine selections and progenies for resistance to blister rust. In: Gerhold, H. D., ed. Breeding pest-resistant trees: proceedings; 1964 August 30–September 11; University Park, Pennsylvania. London: Pergamon Press: 403–414.

Patton, R. F. 1967. Factors in white pine blister rust resistance. 14th IUFRO Congress, Section 22/24: proceedings; 1967 September; München, Germany 3: 876–890.

Patton, R. F. 1972. A brief conspectus of pathology and genetics of *Cronartium ribicola* as related to resistance. In: Biology of Rust Resistance in Forest Trees. NATO-IUFRO Advanced Study Institute: proceedings; 1969 August 17–24; Moscow, ID. USDA Misc. Pub No. 1221: 431–444.

Pei, M. H.; Ruiz, C.; Hunter, T.; Bayon, C. 2003. Rust resistance in *Salix* induced by inoculations with avirulent and virulent isolates of *Melampsora larici-epitea*. Forest Pathology 33: 383–394.

Pierson, R. K.; Buchanan, T. S. 1938a. Age of susceptibility of *Ribes* petiolare leaves to infection by aeciospores and urediniospores of *Cronartium ribicola*. Phytopathology 28: 709–715.

Pierson, R. K.; Buchanan, T. S. 1938b. Susceptibility of needles of different ages on *Pinus monticola* seedlings to *Cronartium ribicola* infection. Phytopathology 28: 833–839.

Poteri, M.; Rousi, M.; Gao, Z.-H. 1997. Differences in the rust resistance of greenhouse and outdoor-grown white birch species, *Betula* spp. Eur. J. For. Path. 27: 363–372.

Rehfeldt, G. E.; Hoff, R. J.; Steinhoff, R. J. 1984. Geographic patterns of genetic variation in *Pinus monticola*. Bot. Gaz. 145: 229–239.

Riker, A. J.; Kouba, T. F.; Brener, W. H.; Patton, R. F. 1953. White-pine trees selected for resistance to white-pine blister rust. In: Seventh Int. Botanical Congress: proceedings; 1950; Stockholm, Sweden: 322–323

Roelfs, A. P. 1988. Genetic control of phenotypes in wheat stem rust. Ann. Rev. Phytopathol. 26: 351–367.

Schjorring, S.; Koella, J. C. 2002. Sub-lethal effects of pathogens can lead to the evolution of lower virulence in multiple infections. Proc. R. Soc. Lond. B Biol. Sci. 270: 189–193.

Sniezko, R. 2002. Some considerations for using major gene resistance to *Cronartium ribicola* in *Pinus monticola* in Oregon and Washington. In: Stone, J.; Maffei, H., eds. 50th Western International Forest Disease Work Conference: proceedings; 2002 October 7-11; Powell River, BC: 54–55

Sniezko, R. A.; Kegley, A. J. 2003a. Blister rust resistance of five-needle pines in Oregon and Washington. In: 2nd IUFRO Rusts of Forest Trees WP Conf.: proceedings; 2002 August 19-23; Yangling, China. Forest Research 16(Suppl.): 101–112.

Sniezko, R. A.; Kegley, A. 2003b. Blister rust resistance experiences in Oregon/Washington: Evolving perspectives. In: Maffei, H.; Stone, J. M. comps. 50th Western International Forest Disease Work Conference: proceedings; 2002 September 9–13; Powell River, BC. USDA Forest Service, PNW, State and Private Forestry: 111–117.

Sniezko, R. A.; Kinloch, B.; Dupper, G. 2001. Geographic distribution of 'Champion Mine' strain of white pine blister rust (*Cronartium ribicola*) in the Pacific Northwest. USDA Forest Health Management National Meetings, 2001. Poster. http://www.na.fs.fed.us/spfo/fhm/posters/posters01/geo.pdf

Sniezko, R. A.; Kinloch, B. B., Jr.; Bower, A. D.; Danchok, R. S.; Linn, J. M.; Kegley, A. J. 2004. Field resistance to *Cronartium ribicola* in full-sib families of *Pinus monticola* in Oregon. In: Sniezko, R.; Samman, S.; Schlarbaum, S.; Kriebel, H., eds. Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance. IUFRO Working Party 2.02.15: proceedings; 2001 July 24–25; Medford, OR, USA. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station, RMRS-P-32: 243–249.

Spaulding, P. 1922. Investigations of the white pine blister rust. U.S. Dept. Agric. Bulletin 957. 100 p.

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

Takken, F. L. W.; Joosten, H. A. J. 2000. Plant resistance genes: their structure, function and evolution. Eur. J. Plant Pathol. 106: 699–713.

Thrall, P. H.; Burdon, J. J. 2003. Evolution of virulence in a plant host-pathogen metapopulation. Science 299: 1735–1737.

van der Gaag, D. J.; Jacobs, Th. 1997. Inheritance of host plant effect on latent period of wheat leaf rust in single-seed descent F8 lines. Euphytica 97: 67–72.

Waters, C. W. 1928. The control of teliospore and urediniospore formation by experimental methods. Phytopathology 18: 157–213.

Williams, N. D.; Miller, J. D.; Klindworth, D. L. 1992. Induced mutations of a genetic suppressor of resistance to wheat stem rust. Crop Sci. 32: 612–616.

Woo, K.-S.; Fins, L.; McDonald, G. I.; Wiese, M. V. 2001. Differences in needle morphology between blister rust resistant and susceptible western white pine stocks. Can. J. For. Res. 31: 1880–1886.

Yanchuk, A. D.; Hoff, R. J.; McDonald, G. I. 1994. Blister rust resistance in western white pine: Does it have a future? In: Baumgartner, D. M.; Lotan, J. E.; Tonn, J. R., eds. Interior cedar-hemlock-white pine forests: ecology and management: proceedings; 1993 March 2-4; Spokane, WA. Pullman, WA: Washington State University Cooperative Extension: 123–132

Yu, G. X.; Braun, E.; Wise, R. P. 2001. Rds and Rih mediate hypersensitive cell death independent of gene-for-gene resistance to the oat crown rust pathogen *Puccinia coronata* f.sp. *avenae*. Mol. Plant Microbe Interact. 14: 1376–1383.

Zambino, P. J. 2000. Evaluating white pine blister rust resistance in *Ribes* after artificial inoculation. HortTechnology 10: 544–545.

Zambino, P. J., and C. H. Michler. 1999. Accelerated identification of eastern white pine families resistant to white pine blister rust. Phytopathology 89: S89.

Zambino, P. J.; Kubelik, A. R.; Szabo, L. J. 2000. Gene action and linkage of avirulence genes to DNA markers in the rust fungus *Puccinia graminis*. Phytopathology 90: 819–826.

Zimmer, C. 2003. Taming pathogens: an elegant idea, but does it work? Science 300: 1362–1364.

Zsuffa, L. 1981. Experience in breeding *Pinus strobus* L. for resistance to blister rust. 17th IUFRO World Congress, Division 2: proceedings; 1981 September 6–12; Kyoto, Japan 2: 181–183.

Zurawicz, E.; Madry, W.; Pluta, S. 1996. Variation and heritability of economically important traits in black currant (*Ribes nigrum* L.) evaluated in a diallel cross design. Euphytica 91: 219-224.