Breeding Rust-Resistant Five-Needle Pines in the Western United States: Lessons from the Past and a Look to the Future

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Abstract—Introduction of *Cronartium ribicola* into the Western United States created major disruptions in forests where five-needle pines were important components. In response, various control measures were implemented on two commercially important species—western white pine (*Pinus monticola*) (WWP) and sugar pine (*P. lambertiana*) (SP). The USDA Forest Service developed three programs to breed for resistance: one directed at northern Rocky Mountain WWP; a second for WWP and SP in Oregon and Washington; and a third for SP in California. The Rocky Mountain program developed a resistant population composed of durable or multigenic resistance that shows no evidence to date of any R genes (i.e., genes for "Major Gene Resistance"). The Cascade WWP program utilizes a mixture of an R gene and multigenes. Washington populations correspond to the Rocky Mountain model, but resistance of the Oregon populations seems to be partly due to an R gene. An R gene is the threshold basis for selection of SP for the California program. Progeny that carry R genes are screened for additional resistance mechanisms to develop populations with multiple sources of resistance. These breeding programs have created large seed banks, several seed orchards, and numerous additional plantings of pedigreed material. However, new concepts for examining genotype by environment (G x E) interactions, recognition of phenotypic plasticity of hosts and pathogens and of induced defenses, and evidence of disease attenuation in the blister rust pathosystem may have important consequences for future breeding, integrated management, and ecosystem restoration efforts in five-needle pine ecosystems. Reanalysis of some old results and update of data from some long-term plantings were coupled with current knowledge of additional pathosystems to suggest an altered paradigm for white pine blister rust in which complex interactions among the pine, rust, and environmental components are the norm.

Key words: Induced defenses, ontogenic resistance, reaction norms, phenotypic plasticity, disease genotype x environment interaction, ecologic restoration

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Introduction

Introduction and spread of the causal agent of white pine blister rust (WPBR), *Cronartium ribicola* J. C. Fischer ex Rabh., in western North America was recently reviewed (McDonald and Hoff 2001). WPBR introduction precipitated a major reduction in populations of western white pine (WP) (*Pinus monticola* Douglas ex. D. Don.) in the northern Rocky Mountains (Neuenschwander and others 1999). In this region, WWP was a modifier keystone species whose presence influenced many ecosystem processes (McDonald and others 2000). Removal of WWP has also altered patterns of mortality from Armillaria root rot on replacement species and thus, of fire regimes that could ultimately produce shortened fire return intervals and more intense stand replacement fires (McDonald and others 2003). Ecosystems where whitebark pine (WBP) (*P. albicaulis* Engelmann) is a keystone species are also at risk of experiencing major perturbations (Tomback and others 2001).

The ecologic and economic importance of North American species of five-needle pines and their susceptibility to WPBR have prompted most genetics work on western five-needle pines. Breeding work has centered on the two important commercial species of WWP and sugar pine (SP) (*Pinus lambertiana* Douglas). Programs were delineated by geographic areas. The first, initiated in 1946, was designed to produce WPBR resistant selections of WWP for use in the "Inland Empire" (northeastern Washington, northern Idaho, and western Montana) and utilized controlled crosses to obtain full-sib progeny for selection within and among crosses (Bingham 1983). A program to develop resistance in WWP and SP for Washington and Oregon was started in 1956. This program switched to screening open pollinated progeny of candidate trees in 1971, after initially using full-sib progeny (Sniezko 1996). A breeding program designed to develop resistance in SP for California was initiated in 1957 (Kitzmiller 1982).

Conditions influencing development of WPBR and types and distributions of resistance mechanisms each vary considerably among these western geographic regions. Differences in approaches among the breeding programs are reflected in differences in kinds of seed orchards and other breeding resources. Our intent in this paper is to briefly describe each program, examine the development, deployment, and historical evidence of effectiveness of resistance to WPBR, and provide insight into the development of management strategies for restoration of ecosystems. Some recent literature will be reviewed. Results in existing

literature will be examined and some previously unpublished results will be presented using new paradigms for interpreting adaptation and epidemiological processes and understanding the physiology of resistance. Finally, we will discuss relevance of lessons learned from breeding WWP and SP to new efforts directed at whitebark pine (WBP), limber pine (LP) (*P. flexilis* James), southwestern white pine (SWWP) (*P. strobiformis* Engleman), bristlecone pine (BP) (*P. aristata* Engelmann), foxtail pine (FP) (*P. balfouriana* Greville & Balfor.), and Great Basin bristlecone pine (GBBP) (*P. longaeva* D. K. Bailey).

The Breeding Programs

Northern Rocky Mountain Western White Pine—This breeding program has had two phases. The first phase was based on about 400 phenotypically resistant (canker-free to five cankers/tree) selections obtained from areas of high rust severity (cankers/tree). This phase was initiated in 1946 when R. T. Bingham selected a full-crowned 60-year-old tree almost 30 meters tall as the only rust-free tree within a northern Idaho stand of 380 trees (Bingham 1983). By June of 1950, 58 trees had been selected from similar areas of high rust severity. Counts of cankers from neighboring susceptible trees at the time of selection (data on file at the Rocky Mountain Research Station, Moscow, Idaho) allowed infection rates and probability of escape to be calculated. Under the levels of rust incidence and severity observed for stands containing the 58 selections, only one uninfected tree out of 143 million would be expected to be an escape (McDonald and Hoff 1982). The full contingent of 400 candidate trees had been located by 1970 and the ratio of resistant (rust-free to five cankers per tree) to susceptible phenotypes in natural populations was established at 0.00001 (McDonald and Hoff 1982).

Beginning in 1950, selections were crossed to produce full-sib families whose seedlings could be artificially inoculated to measure general combining ability (GCA) and specific combining ability (SCA) (Bingham 1983). Wild inoculum from two to three collection sites was used for each inoculation. Inoculations were conducted under a double-layered tent (Bingham 1983). One fourth of the 400 parent trees selected as phenotypically resistant proved to have GCA for enhanced rust resistance. These 100 parents were ascribed to three populations corresponding to elevation, with the "low elevation" class originating below 1065 m, the "mid-elevation" class from 1066-1250 m, and the "high elevation" class from over 1251 m. Since a balanced design of crossing was desired and the smallest elevation class contained 24 GCA parent trees, 24 GCA trees were selected to represent each elevation in subsequent controlled crosses. Foundation stock for each of the three F_2 seed orchards representing the three elevation classes was of 12 unrelated GCA x GCA families. Early artificial inoculation tests indicated that about 65 percent of the seedlings of GCA x GCA F_2 families remained free of infection after intense rust exposure (Hoff and others 1973).

The Phase I program was noted for selection of candidate trees from stands under intense rust pressure that were then used in various crossing schemes (Bingham and others 1969). Full-sib progeny were subjected to artificial inoculation in tests containing many thousands of seedlings (Bingham 1972). Phase I generated many peer-reviewed papers and left a legacy of nine well-documented plantations and four seed orchards (Mahalovich and Eramian 1995). Several are composed of well-marked pedigreed materials suitable for new genetic research (records on file USFS Region 1). A recently accepted paper uses these historical records and materials to analyze the influence of blister rust resistance breeding on the genetic structure of WWP as revealed by AFLP DNA markers (Kim and others 2003). Several plantings of Phase I materials have received repeated rust examinations and new data obtained from these plantations will be discussed later in this report. The Phase I program was also the source of descriptions of most of the resistance mechanisms (see Hoff and McDonald 1980 for descriptions) that have since been applied to the ongoing breeding programs in the northern Rocky Mountains (Mahalovich and Eramian 1995) and Oregon and Washington (Sniezko 1996).

Northern Rocky Mountain WWP—Phase II Program—Region 1 of the USDA Forest Service and the Inland Empire Tree Improvement Cooperative (see Fins and others 2002) administers the Phase II breeding program that was initiated in 1967 (Mahalovich and Eramian 1995). This program is based on open pollinated (OP) selections screened for inheritance of a set of resistance mechanisms (Hoff and McDonald 1980, Mahalovich and Eramian 1995). The objective is to select for resistant progeny among seedlings of 3100 candidate WWP that have been chosen from stands at least 25 years in age. Maximum severity (cankers/tree) acceptable for select trees is set relative to severity in the surrounding stand as follows: 0 if severity was 10 to 20, 1 if severity was 21 to 40, 2 if severity was 41-75, 3 if severity was 76-150 and 4 to 5 if severity was 150+. Severity on most selected trees was less than three cankers. OP cones have been collected from about 200 candidates each year for screening at the USFS Region 1 Tree Nursery, Coeur d'Alene, Idaho. Seedlings are grown under standard nursery regimes for inoculation at the end of their second growing season.

Inoculum is generated in a disease garden located at the Lone Mountain Tree Improvement Site, Spirit Lake, Idaho. Aeciospores are collected each year at 10 sites covering a target-breeding zone that includes the Idaho panhandle and western Montana (Mahalovich and Eramian 1995). Collected spores are used to initiate a controlled epidemic on *R. nigrum* L. and *R. hudsonianum* var. *petiolare* (Dougl.) Jancz. Telia-bearing leaves are collected and transported to the Coeur d'Alene nursery for use in controlled inoculations as had been done for the Phase I program. Following September inoculations, seedlings are planted in beds outdoors and inspected the following June for numbers of needle lesions and the following September for presence/absence of needle lesions, bark reactions, and cankers. The second and third September after inoculation, seedlings are inspected for presence/absence of bark reactions. Data are used to select both families and individuals within families in an attempt to accumulate resistance mechanisms (Mahalovich and Eramian 1995). Families selected from the Phase II program as having high proportions of rust-free seedlings at the fourth inspection are rigorously outplanted to evaluate long-term rust behavior and growth under operational conditions and natural levels of inoculum (Mahalovich and Eramian 1995). Over 20 plantings have been established,

using sound statistical designs, and plans are in place to conduct regular inspections (Mahalovich and Eramian 1995). Survival, damage to terminal stems, presence/absence of bole and branch cankers, number of stem cankers, number of branch cankers, and total tree height will each be tracked. The planned assessment schedule is 5, 7, and 10 years after planting, followed by 5-year intervals until one half of the rotation age has been reached (Mahalovich and Eramian 1995).

Three additional testing regimes have been implemented in the Phase II program. A crossing study with full-sibs and selfs was initiated in 1993 to verify the genetic mechanisms and inheritance for needle shed, short shoot, and bark reaction traits, with crossing taking place at two field sites and the Coeur d'Alene nursery. Secondly, realized gain trials have been designed to test resistance of seed orchard populations from both Phases under operational conditions. For this study, F_1, F_2, B_1 and several control lots will be outplanted in 2004 at sites where infection levels have been increasing in plantations of resistant material. Lastly, four tests, each replicated at two locations per year beginning in 2001, are being planted to evaluate genotype by environment interactions and the idea that a single breeding zone is sufficient for the northern Rockies. Results of the new testing regimes will facilitate validation or revision of the breeding strategy.

Additional breeding was initiated in 1995 using elite tree selections from the Phase I and Phase II programs to generate a second-generation program and obtain further potential gains in resistance. Up to 360 selections will be divided into 18 sublines that will ensure that seed from the 2^{nd} generation program will minimize the potential for inbreeding. Also, a WWP clone bank was established in 1999 at Dry Creek Tree Improvement Area, Clark Fork, Idaho to protect unique trees, elite trees, and progeny of candidate trees in the field that have been lost through timber removal, road construction, and catastrophic fire. Entries are added annually.

Oregon and Washington Sugar Pine and Western White Pine—The Washington and Oregon breeding program is operated out of the Dorena Genetic Resource Center, USFS, Region 6 and is located near Cottage Grove, Oregon (Sniezko 1996). The USDI Bureau of Land Management, Oregon Office, has been a major cooperator, particularly for sugar pine, in the phenotypic selection of trees and development of seed orchards for many years. Other cooperators represent a wide array of public agencies, Indian nations, and other organizations.

Objectives of the resistance-breeding program for Oregon and Washington include identifying the amount and type of genetic resistance present in natural populations of WWP and SP, selecting families and individuals within families for resistance, and developing durable resistance to WPBR while retaining broad genetic diversity and local adaptation within both species. The program utilizes conventional breeding techniques to increase the durability of the partial resistance currently available, while maintaining diverse genetic populations. Patterns of genetic adaptation in SP and WWP identified through growth responses in common garden studies (Campbell and Sugano 1987, 1989) are the primary basis for delineation of breeding zones.

The Dorena facility has screened seedlings of WWP or SP from more than 9500 parent trees. The program has the capability to inoculate more than 600 families per year and evaluate over 100,000 seedlings annually with consistently high seedling infection rates, as evidenced by development of needle lesions and stem symptoms. Inoculations are performed in a building that has been fitted with humidifiers and associated controls to act as a large, self-contained incubation chamber (McDonald and others 1984). Seedlings are inoculated after their second growing season and are evaluated for five years thereafter to discern different resistance mechanisms. Historically, this program has used the same set of definitions of resistance as the northern Rocky Mountain Phase I and Phase II programs (see Sniezko 1996), although some re-evaluation of mechanisms is underway. In general, 80 to 240 (often 120) families are inoculated per 'run', with each family represented by 60 seedlings divided among six replications. Inoculum comes from two sources: collections of telia produced in the wild from natural inoculum under ambient conditions vs. in a disease garden under more controlled conditions.

Until recently, conditions and techniques used in inoculation and evaluation of most forms of resistance had changed only slightly since the 1960s. However, Dorena has recently begun to use a modified screening technique to differentiate families expressing an R gene (*Cr2*) specific to WWP (Kinloch and others 1999) that develops a hypersensitive reaction as a barrier to colonization in infected needles from other families that have a high incidence of canker-free seedlings. Previously, disease-garden telia were generated after *Ribes* were inoculated with bulked collections of aeciospores obtained from across Oregon and Washington. Currently, *Ribes* in the disease garden become naturally infected by aeciospores from WWP in the general vicinity. Teliospore-bearing leaves from the disease garden are used to represent a locally prevalent, virulent strain of rust (the "Champion Mine Strain") that can overcome *Cr2* resistance (McDonald and others 1984, Kinloch and others 1999). Since wild-collected (wild-type race) and disease garden (virulent race) *Ribes* leaves are each used in half of the inoculation replications, differences in frequency of resistance between inoculum sources identify resistance of families conferred by the R gene, and differentiate them from forms of resistance (partial resistance) that are not known to be overcome by differences in rust race. Results from such inoculations indicate that in Region 6, the frequency of the WWP R gene is very low and that existing levels of partial resistance are low, although a few outstanding parents exist (Kinloch and others 1999, Sniezko 1996, Kegley and Sniezko this proceedings).

Recent re-sowing of some previously tested families has confirmed their resistance. Demonstration plantings at Dorena that include representatives of such resistant families alongside susceptible families that develop extensive cankering and mortality provide a dramatic visual demonstration of the effectiveness of the program to visitors. Tentative discovery of a new resistant mechanism, termed "mechanism X", may cause further changes to the program. One of us (RS) has observed that "X" is not *Cr2* because of resistance to the *vcr2* race of the pathogen and that "X" families do better than *Cr2* families in field tests; one of us (GM) has also observed that needle shed morphology of "X"

is reminiscent of Idaho WWP needle shed. Only Washington populations of WWP have been shown to express "X" but further investigations of Oregon populations are underway (Sniezko unpublished data).

Much of the early activity in the Oregon-Washington white pine blister rust (WPBR) resistance program involved selecting trees in natural stands in each of eight breeding zones, inoculating and evaluating offspring of these trees for resistance, and establishing seed orchards from the most rust-resistant parents or progeny. Seed orchards have been established for both WWP and SP in most breeding zones and resistant seed is available for several zones. Orchards include a diverse set of parents and an array of putative resistance mechanisms.

Recently, an important, but long neglected, component has received additional attention. Establishment of field plantings is now under way to monitor resistance, validate screening results, and demonstrate the potential for resistant seed in ecosystem restoration throughout Oregon and Washington. Since 1996, families of WWP and SP have been established at 20 and 10 sites, respectively, in conjunction with cooperators that include USDA Forest Service Region 5, BLM, Confederated Tribes of Warm Springs, OR, and Josephine County, OR. Additional trials, using control crosses or open-pollinated seedlots from Dorena, have been established in British Columbia and on WA Department of Natural Resources lands (Rich Hunt, personal communication 2001). A planting in the Oregon coast range is planned by Plum Creek Timber Company (Jim Smith personal communication). Full sib or OP families are utilized and have been established at sites that differ in representation of the presumed races of blister rust. These plantings will help establish whether there are changes in rust virulence over time, and will potentially give information on inheritance of some of the resistance mechanisms (for example results, see Sniezko and others 2000, Sniezko and others, these proceedings). Information from a few older plantations has also recently been collected, summarized, and presented at technical meetings (Sniezko and others #2, these proceedings). In these older trials, infection levels after 15 or more years in the field are high (>75 percent of trees infected). One goal of these plantings is to determine the relative field performance of WWP and SP lots that differed in levels of rust resistance during screening, to calibrate by species and resistance level the WPBR extension (McDonald and others in preparation) of the Forest Vegetation Simulation Model (Teck and others 1997) for use in Region 6.

California Sugar Pine—A program designed to identify and utilize WPBR resistance in California sugar pine was initiated in 1957 (Kitzmiller 1982) and is conducted out of facilities located near Placerville, CA. The goal of this portion of the USDA Forest Service Region 5's genetics program is to maintain locally adapted SP having a mixture of resistance mechanisms throughout sugar pine's natural range (Samman and Kitzmiller 1996). This is being achieved through two approaches. First, resistant trees are identified that can be used as seed sources for each of 27 California seed zones (Kitzmiller 1976) where sugar pine occurs, with trees additionally grouped according to 500 ft altitudinal bands. Secondly, a subset of these and additional selected resistant materials are being used to establish seed orchards and breeding populations for each of seven breeding zones that have been established based on physiographic and environmental parameters (Samman and Kitzmiller 1996).

Criteria for tree selection vary according to geographic location and the frequencies at which resistance mechanisms occur within local populations. The first-level criterion for seed tree selection is the presence of the sugar pine R gene (*Cr1*; Kinloch and Davis 1996). This was the first dominant gene for resistance identified in a forest tree species (Kinloch and Littlefield 1977), and like *Cr2* described above, restricts needle colonization and stem infection by *C. ribicola* by development of a hypersensitive response (Kinloch and Littlefield 1977). Assessment is based on symptom development in inoculated progeny seedlings. In regions where frequency of the R gene, and consequently, the occurrence of disease free sugar pine are sufficiently high, timber and growth traits are also considered. Conversely, in regions such as the northern part of the range of sugar pine where the R gene for sugar pine is low in frequency (Kinloch 1992), initial tree selection is based solely on freedom of trees from disease in stands with high incidence of blister rust. Selections are protected from cutting until screened.

Open pollinated seed is collected and progeny seedlings screened for the R gene at the Placerville facility using artificial inoculations. Control lots are included that contain several representative genotypes homozygous or heterozygous for *Cr1* or homozygous for lack of the R gene. Telia used as screening inoculum are produced by inoculating greenhouse-grown plants of susceptible cultivars of *Ribes nigrum* L. with aeciospores of rust that lacks virulence to *Cr1* that have been collected from sugar pine in different areas, or with urediniospores produced after previous inoculations. Inoculated plants are bagged for 24 hours until infection has occurred, and then maintained in a cool, partially shaded greenhouse for four to five weeks until dense telia develop on leaves. Basidiospores are cast from telia on trays of detached leaves onto 8-month-old seedlings in three-layered, cloth-lined chambers in which temperature and humidity are kept at optimum levels for infection by water flowing over the outer walls. Seedlings are each evaluated three times approximately three weeks apart after susceptible yellow or yellow-green needle spots can be distinguished from necrotic spots or yellow spots with necrotic margins that signify resistance due to the sugar pine R gene. Open pollinated families with approximately 50 and 100 percent resistant seedlings indicate heterozygous and homozygous parent trees, respectively, whereas lower proportions indicate a non-*Cr1* tree that has received pollen from one or more *Cr1* trees.

Those seedlings with the R gene are further screened for slow rusting under natural conditions at a location (Happy Camp, California) where a strain naturally occurs that can overcome the sugar pine R gene (Kinloch and Davis 1996; Samman and Kitzmiller 1996). *Ribes sanguinium* Prush. is planted among the R-gene-resistant seedlings to produce natural inoculum having a high proportion of *vcr1* virulence to the sugar pine R gene resistance. Seedlings are exposed to a minimum of two, and usually three "wave" years of heavy rust infection, with final slow rust determinations made for seedlings and families a minimum of ten years after planting. Detection of slow-rusting resistance at the Happy Camp site is based on observable low rates of increase in rust incidence and severity, slow or abortive colonization, and

reduced persistence of infections. Normal cankers and bark reactions (corking out of branch and bole infections) in SP appear similar to those in WWP in the other resistance programs, although the Region 5 sugar pine program also differentiates a "blight" reaction in which necrotic tissue extends beyond the canker to the end of the branch proximally to the next branch whorl and distally to the end of the branch (Kinloch and Davis 1996). Materials selected at Happy Camp for having slow-rusting resistance in addition to R gene resistance are then grafted and planted into seed orchards and clone banks.

To date, 28654 candidate trees have been selected; 1395 living MGR trees identified (John Gleason, R5 Genetics, pers. comm.); and approximately 2900 pounds of MGR seed is available for restoration efforts. The three seed orchards closest to completion represent the Sierra Nevada (i.e., Breeding zones 4, 5,and 3, in order of completeness). Each of these orchards has separate but adjacent breeding blocks for R gene only versus R gene plus slow-rusting resistance mechanisms (durable resistance), and additional divisions based on geographic and elevation differences. Duplicate plantings of some of this material are being developed as seed orchards by cooperators in industry, providing a buffer against loss by fire or other causes. Although the numbers of R-gene parent trees needed for the Sierra have been met and exceeded (currently over 700 represented in the orchard design), adequate numbers of selections have been difficult for some extremes of elevation and for the northern range of sugar pine. To increase the number of R-gene phenotypes from such locations, the program is also identifying resistant MGR progeny of phenotypically resistant seed trees in the northern range that lack the R gene. Lack of the R gene in these phenotypically resistant trees may indicate high levels of non-MGR resistance mechanisms. Fertilization by R gene pollen may thus result in offspring that have several resistance mechanisms. Pollen receptor, R-gene-carrying progeny that are being used in the program are geographically distant from known R-gene trees, to maximize genetic diversity. Alternative methods are being investigated that may enable north zone candidate trees with potentially high slow-rusting resistance to be identified during the initial progeny screening for R gene resistance.

A network of widely dispersed plantations has been established to evaluate silvicultural growth characteristics and resistance of SP to both the wild type and the *Cr1*-virulent races of WPBR, (Kitzmiller and Stover 1996). Eight plantations established in 1983 utilized the same families at each site, and included families that were homo- and heterozygous for *Cr1* and susceptible families. Four plantations established in 2000-2001 include homo- and heterozygous *Cr1* and susceptible families, as well as R gene plus slow-rusting families. Within each category, some families have demonstrably wide geographic adaptation, while other families are of local origin (P. Stover, personal communication). Plots are monitored primarily for incidence of disease and presence and numbers of cankers on branches and stems. Thus far, monitoring after wave years has failed to detect the race of WPBR virulent to the sugar pine R gene outside of the immediate vicinities of its two known sites of occurrence at Happy Camp and Mountain Home, California.

Other Five Needle Pine of the Western United States—Some breeding work for other five needle pines is just getting underway (Mahalovich and Dickerson these proceedings). A common garden study designed to delineate seed zones for northern Rocky Mountain WBP was planted at the Priest River Experimental Forest in 2000 (D. Ferguson, personal communication). Open pollinated seed, collected from 10 trees in each of 45 stands, was planted in 2000. Growth, development, and periodicity of shoot elongation will be monitored using data collection methods patterned after a previous WWP study (Rehfeldt and others 1984). A second planting of most of the same sources was installed in 2001 on a dry site at the Priest River Forest to study drought tolerance of WBP (Ferguson personal communication). Seedlots from a number of national forests in OR and WA were also included in a recent common garden study initiated at the University of British Columbia (Andy Bower, personal communication). These studies should provide insights into adaptive variation in WBP.

Evaluations of a small number of California WBP seedlots for R genes (Kinloch and Dupper 2002) and OR/WA WBP (unpublished data) have failed to find them. However, R genes were only recently confirmed in LP and SWWP (Kinloch and Dupper 2002). A recent sowing of WBP at Dorena will increase the number of parents evaluated in Region 6 for R genes and other resistance responses. Protocols will be adapted from the Region 6 WWP and SP resistance program and other efforts to screen for resistance in WBP (Hoff and others 2001). Spore load for evaluation and comparison of WBP sources will be a critical factor. An inoculation trial in 2001 at Dorena has identified the spore load needed for infection of this species under local conditions (Kegley and others in press). A recent preliminary screening of phenotypically resistant WBP growing in the northern Rocky Mountains demonstrated a resistance signature (low frequency and proportion of spotting among progeny seedlings) very similar to that of northern Rockies WWP (Hoff and others 2001). Early in the development of WPBR in western North America, a study quantitatively compared WPBR incidence and severity on WWP and WBP at six sites in Washington, Oregon, and Idaho (Bedwell and Childs 1943). Their data were used to compute infection rates using the "monomolecular" or "monocyclic epidemic" equation (see below) in WWP and WBP (McDonald and Hoff 2001). Comparison of these rates indicated WBP growing in the northern Cascade Mountains of Washington and in the northern Rocky Mountains in northern Idaho were about 70x more susceptible than contemporary WWP, while WBP populations in the vicinity of Mount Hood, OR were only 4x more susceptible (McDonald and Hoff 2001). Genetic differentiation of populations was revealed as an explanation for differences in relative susceptibility by a recent study of genetic structure of WBP (Richardson and others 2002). This study demonstrated distinct southern and northern populations of WBP that exhibited a distinct zone of hybridization between Bedwell and Childs' (1943) Mount Hood and northern Washington Cascade populations of WBP.

New Looks at Historic Issues

Separating Fact from Fiction—The WPBR pathosystem has been in North America for almost a century; intense efforts to control the disease by minimizing the

alternate host had been applied; and we have 50 years of experience in developing resistance. Yet, detection of multiple forms of resistance, virulence that overcomes one form of resistance in several hosts, observations on incidence and severity of infection, and behavior of local epidemics all lead us to ask critical questions of the breadth of our knowledge of this system. Have we examined the blister rust pathosystem in sufficient detail to predict whether disease in stands genetically improved for resistance will perform as predicted by our past experience with the epidemic on susceptible plants, by artificial screening results, or by models that predict disease incidence over time and space? Have important assumptions about WPBR behavior remained unchallenged? Can new tools be employed to critically measure WPBR pathosystem dynamics in time and space? What is the relative importance of classic genetic trait-oriented selection vis-à-vis other sources of genetic expression and change?

R genes and Resistance-Virulence Interaction—The classic gene-for-gene system seems to play an important role in WPBR. A series of papers has demonstrated that SP, WWP, SWWP, and LP have R genes (major genes for resistance) at some frequency in their populations (Kinloch and Comstock 1980, Kinloch and others 1999, Kinloch and Dupper 2002). Two virulence genes (*vcr1* and *vcr2*) are known that negate the resistance conferred by R genes in SP and WWP, respectively. Virulence genes have not been found that negate R genes in SWWP and LP, indicating that the three genes are distinct (Kinloch and Dupper 2002). At least one variant of *C. ribicola* is virulent on both SP and WWP R genes (Kinloch and Dupper 2002). Some evidence indicates that the *C. ribicola* gene for virulence to the sugar pine R gene may be inherited by way of the cytoplasm (Kinloch and Dupper 1999). The WWP R gene has not been found in Northern Rocky Mountain populations but is found in the Oregon Cascade Mountains (Kinloch and others 1999), with additional occurrence in the northern Sierra (Kinloch and others, unpublished). In some cases, expression of the WWP R gene seems to be subject to maternal influences, which may complicate its recognition (Kinloch and others 1999). Distribution of the virulence gene that overcomes the WWP R gene was tested using teliospore samples collected for seedling inoculations, and was identified as prevalent in recent years at Champion Mine (CM) and Grass Creek (GC) (Kinloch and others 1999, Kinloch and Dupper 2002) in the Oregon Cascade Mountains. Presence of the virulence gene was signified by the development of typical susceptible interactions in a group of full-sib families from Champion Mine that carry the WWP R gene (Kinloch and others 1999).

Inoculations of some of these same full-sib WWP families with rust collected in different ways at both CM and GC in 1984 and 2000 demonstrated an effect of *Ribes* source (or environmental aspects correlated with occurrence of *Ribes* species) and disease development. In their analysis, McDonald and others (1984) used three different sources of CM inoculum: telia collected directly at CM from *R. sanquineum* or *R. bracteosum* (Dougl.), and telia produced on *R. bracteosum* at Dorena, Oregon from aeciospores collected from WWP at CM (McDonald and others 1984 table 3). The most striking difference among these sources was the 4X higher rates of rust incidence (needle lesions) on WWP per equivalent basidiospore density when telia had been produced *in situ* at CM on *R. bracteosum* vs. the nearby *R. sanquineum*. Telia produced at Dorena from CM aeciospores behaved like the telia developed at CM on *R. bracteosum* except that it took significantly longer to kill seedlings so that significantly more stunted leaders of living plants were noted at the time of assessment (McDonald and others 1984). These results strongly suggest that growth on different *Ribes* species at this location provides an important G x E effect on ability of *C. ribicola* inoculum to subsequently infect and develop on pine. These differences may be mediated through differential ability of a virulent race to utilize local *Ribes*, or through more subtle interaction. Even though WPBR from the GC site may now carry *vcr2* virulence (Kinlock and others 1999), in 1984 its apparent lack of virulence to the WWP R gene was similar to WPBR obtained at the Still Creek (SC) site (see McDonald 2000) in its apparent lack of virulence to the WWP R gene (McDonald and others 1984). However, the GC inoculum produced significantly more cankers that were delayed in their development after needle infection than did the SC inoculum (McDonald and others 1984).

Ontogenic Resistance—An increase in ability of plants or plant parts to resist a pathogen as they age and mature and the physiological mechanisms by which this originates are well documented in agricultural pathosystems (Ficke and others 2002). However, in agricultural systems, plant development is generally restricted to a single season. The situation is much less clear for pathosystems involving long-lived hosts that nonetheless have yearly production of foliage and stems. Two types of ontogenic resistance have been described for WPBR: resistance related to types and maturity of needle tissues, and resistance related to physiological aging or other phenomena correlated with tree age.

Seedlings of the five-needle pines pass through four distinct maturation phases. Seeds germinate and produce cotyledons, primary (simple) needles, and occasionally secondary or fascicled needles during their first growing season. In most seasons in subsequent years, only secondary needles are produced, and these may be retained for multiple seasons. However, under certain environmental conditions, even older seedlings may produce primary needles in varying numbers, as part of a variant shoot morphology known as "lammas growth".

Cotyledons and primary needles are extremely susceptible to infection and colonization and have been used for early screening of R gene resistance in SP where resistant and susceptible spots can be visually differentiated (Kinloch and Comstock 1980). These juvenile needles have also been used experimentally to assess rates of seedling colonization and subsequent mortality in families of eastern white pine (EWP) (*P. strobus* L.), since infection of both primary needles and stems of such immature plants is uniformly high (Zambino unpublished data). Conversely, the Phase I WWP resistance-breeding program relied on the differential between needle lesions and stem canker incidence to signify resistant families. An attempt to cut costs by inoculating seedlings at the end of their first growing season nearly eliminated the differentiation between resistant and susceptible seedling families (Bingham and others 1969). A large-scale inoculation of WWP subsequently confirmed that significant difference in needle lesion and canker incidence was associated with inoculation age and family

(Bingham 1972). The same pattern was observed in an interspecific cross of EWP with an Asian pine presumed to have a well-evolved resistance to WPBR. Patton and Riker (1966) inoculated two families of EWP x Himalayan white pine (*P.griffithii* McClelland) hybrids at 4.5 months and 48 months, and obtained canker incidences of 0.997 and 0.4 respectively.

Ontogenesis and maturity of individual needles also has a strong influence on resistance; lack of appreciation for this fact may explain variability in screening success and in ability to model the response of selected materials under natural conditions that have repeated opportunities for infection, versus controlled inoculations that are generally exposed at a single time. In inoculation experiments, "year old" secondary needles of EWP produced 3.7 times more infection per linear distance of needle than "fully mature current year" needles (Hirt 1938). In similarly inoculated WWP, the ratio between "year-old" and "current year" needles was 14.5 to 1 based on needle area (Pierson and Buchanan 1938). Most screening includes infection data on needles within or at the end of the first year of development, even though no studies have shown how this practice might influence field performance of selected materials or the expression of canker resistance mechanisms. Timing for inoculation within the current year needle development may also be critical as inoculation of WWP secondary needles before they have completed development circumvents the expression of resistance in needle tissues (Woo 2000). In addition, stomatal density and shape and contact angle of water droplets on needle surfaces showed significant differences in comparisons of resistant and susceptible WWP (Woo and others 2001 and these proceedings).

Finally, branch and stem resistance appears to increase with age: Comparisons among grafts obtained from susceptible and resistant ortets of various ages and susceptible seedlings demonstrated that resistance increases with increasing age of the scion source for both resistant and susceptible grafts of EWP (Patton 1961). In addition, the act of grafting alone reduced incidence from .99 to .81 in 4-year-old seedlings (Patton 1961). In SP exposed to rust in the Happy Camp disease garden, average incidence among grafts of 17 ortets was 0.32, whereas seedling families obtained from the same 17 candidates showed an incidence of 0.92 while susceptible control seedlings had an incidence of .98 (Kinloch and Byler 1981). This apparent increase in a tree's resistance with age must nonetheless be reconciled with the fact that shortly after the introduction of WPBR, canker incidence in some merchantable WWP stands reached 100 percent and was followed by high levels of mortality (Buchanan 1936), indicating the ineffectiveness of the extant levels of ontogenic resistance at that time.

Ontogenic or Induced Resistance?—Defenses against most enemies in most living organisms may be either constitutive or induced (Fluhr 2001). Most animals have both kinds of defense, while plants may have evolved mostly constitutive defenses in the form of R genes that stand as sentinels on the lookout for virulent pathogens (Fluhr 2001). These R genes have certain molecular signatures found across many plant taxa (Fluhr 2001). Some of these signatures have been observed in both SP (Kinloch and Dupper 2002, Sheppard and others 2000) and north Idaho WWP (Liu and Ekrammoddoulah 2004; M.-S Kim personal communication). Perhaps these signatures are a sign of the widespread occurrence of R genes in the five needle pines as suggested by Kinloch and Dupper (2002).

Other recent reports suggest that induced defenses might also be widespread in plants as well as animals. In the case of the desert locust, *Schistocerca gregaria*, increased population density induced increased resistance to bacteria and fungi (Wilson and others 2002). A large and rapidly developing literature is available for the topic of inducible (epigenetic) defenses in plants and animals that we will not attempt to present here. Induced defenses vary from morphologic plasticity able to ward off predation or infection to induced physiological resistance (Tollrian and Harvell 1999). Epigenetic R-gene resistance that is expressed in subsequent generations was demonstrated in *Arabidopsis thaliana* (L.) Schur (Stokes and others 2002).

Since the topic of inducible defenses impinges on many aspects of WPBR resistance breeding, it should be watched closely. Impacts could range from understanding ontogenic resistance through examination of phenotypically resistant candidates, to improved selection and interpretation of performance of "control" lots used in progeny screening tests and field plantings. Inducible defenses have already been reported in both native (evolutionarily well-established) conifer pathosystems such as fusiform rust-loblolly pine (Enebak and Carey 2000), and Norway spruce (*Picea abies* (L.) Karsten)-blue-stain-(*Ceratocystis polonica*) (Krokene and others 2001, Franceschi and others 2002), as well as recently established interactions such as Monterey pine (*P. radiata* D. Don.) — pitch canker (*Fusarium circinatum*) (Bonello and others 2001). Also, stilbenes — effective conifer phytoalexins — are induced in various plant parts including primary needles of Scots pine by such diverse challenges as fungal attack in the phloem, UV light, and stress (Chiron and others 2000).

Evidence for Induced Resistance to WPBR—In WWP, 36 phenotypically resistant ramets (clonal offspring) that had been obtained by grafting from select trees from the field (ortets) were established in plantations and exposed to levels of field inoculation that produced 0.89 incidence of cankering of control seedlings at the same sites after 13 years of exposure (Bingham 1966). Full-sib offspring were obtained from 34 of the ortets that produced the tested ramets. Controlled crosses were made on the 34 seed parents using a common set of four pollen donors. When 11744 seedlings of these families were subjected to artificial inoculation, canker incidence was 0.84 (calculated from table 4, Bingham 1966). Comparisons between infection of the ortets (plants in the field) and their ramets should indicate presence or absence of induced resistance. Bingham (1966) noted that ramets of all nine of the ortets that were infected when selected did not themselves become infected; however, five of the 25 ortets that were uninfected when selected had ramets that became infected. Three kinds of phenotypically resistant parents (ortets) are suggested. First, those in which a few cankers induced resistance that prevented subsequent infection of their ramets (i.e., the 9 infected ortets). Second, those that may not have been resistant or may have been capable of induced resistance that was not triggered (i.e., the 5 ortets whose ramets became infected). The third class is composed of those trees that possessed putative constitutive resistance

(an additional 20 rust-free ortets that produced rust-free ramets). This situation implies induced resistance. Furthermore, comparison of performance of seedling progeny of the three classes may indicate the first evidence of transgenerational induction of resistance in conifers: Log-odds ratios (Sokal and Rohlf 1995) indicated significant differences in rust incidence on seedlings that differentiated the groups from one another. Rust incidence was lowest (0.81) among 6863 seedlings representing the 20 "constitutive resistance" ortets, highest (0.92) among the 1612 seedlings representing the 5 "uninduced" ortets, and intermediate (0.88) among 13269 seedlings representing the 9 "induced" ortets.

G x E in Plantations of Rust Resistant WWP—Over the years, WPBR-resistant stocks developed in the north Idaho Phase I program were planted and/or inoculated in many different environments. Differential responses in results illustrate gaps in our knowledge concerning the relative contributions of environment, rust genes, alternate host diversity, pine diversity, and resistance genetics to the behavior of WPBR. The most interesting of these tests involve long-distance transfer of materials. For example, susceptible controls and several full-sib F_1 and F_2 families from the Phase I program were subjected to natural and artificial inoculation at two sites in Japan where *C. ribicola* basidiospores are produced on a host (*Pedicularis resupinata* L.) in the Scrophulariaceae (Yokota 1983). The overall conclusion was that resistant material developed in north Idaho was not resistant to the rust in Japan. Stock from the Phase I program was also planted at several sites in British Columbia (Bower 1987, Hunt and Meagher 1989). In a direct comparison after 11 years at a low elevation site (Mesachie), disease incidence was higher (.75) for the Phase I F_2 than for local natural unimproved stock (0.52) (Hunt and Meagher 1989). The pattern was reversed at a second site 45 km north of Mesachie at Northwest Bay where a higher elevation site produced incidences after 13 years of 0.12 for the F_2 vs. 0.40 for local stock (Hunt and Meagher 1989). These authors describe further significant G x E interactions for several sources of resistant WWP as well as EWP. Significant G x E constitutes a warning to pay close attention to inoculum sources and geographic distributions of materials selected for resistance. G x E was also observed in field plantings of slash pine (*P. elliottii* Englm var. *elliottii*) resistant to *C. quercuum* (Berk.) Miy. ex Shirai f. sp. *fusiforme* (Schmidt and Allen 1998). Significant G x E in rust behavior signifies that geographic partitions in addition to simple breeding zones may be a required strategy in restoration programs.

New Tools and Concepts

Comparative Epidemiology—Plant disease epidemiologists have focused on modeling and disease management from an endpoint perspective, while medical epidemiologists have focused more on elucidating component parts, enabling them to act as disease detectives who can reconstruct the whole by understanding the interactions of the parts (Waggoner and Aylor 2000). Subsequent to the inception of pine breeding programs in western North America, advances in epidemiological theory and evolutionary biology have added several tools that should enhance our own capability to become good disease detectives and to focus on how the various parts of WPBR interact. Successful breeding and deployment of resistance in a long-lived pathosystem requires significant understanding of how epidemics function across time and space, which can lead to better integrated management of blister rust and restoration of ecosystems dominated by five needle pines.

Rust Progress Curves—Measuring disease is a significant problem often approached by determining incidence (proportion of a population of plants or plant parts infected) and severity (number of lesions or amount of area of plants or plant parts infected) periodically during the progress of the epidemic and estimating curves from the plotted values. Nonlinear disease progress curves and their associated linear incidence rate equations (Madden and Campbell 1990) can be useful for comparing epidemics and predicting outcomes in new times and places. Fracker (1936), the first to apply nonlinear progress curves to plant disease epidemics, was also first to apply them to WPBR. Since then, epidemiological theory has matured into several straightforward concepts (Zadoks and Schein 1979, Madden and Campbell 1990). Almost all epidemics are described by one of two basic forms. For most diseases and environments, epidemics behave as a polycyclic process where diseased plants contribute inoculum to increase local disease within the same growth cycle. Such epidemics are represented as a logistic curve where the inflection point indicates the point at which the acceleration in rate of disease increase due to increased inoculum is offset by the decline in healthy tissues available for colonization. Other epidemics behave as a monocyclic process where local diseased plants do not contribute to additional disease because inoculum is from relatively constant external sources. Because amounts and infection efficiency of inoculum are relatively constant and not affected by local feedback, curves that describe this process have no inflection point. This "monomolecular" disease progress curve and its associated absolute infection rate have been applied to WPBR (Kinloch and Byler 1981, McDonald and Hoff 1982, Goddard and others 1985, McDonald and others 1994, McDonald and Hoff 2001). Presence or absence of an inflection point may provide important clues about fundamental disease processes such as relative importance of local vs. long-distance spread of inoculum and degree of rust multiplication on alternate hosts.

The epidemic asymptote K indicates maximum incidence in an epidemic (Madden and Campbell 1990). The idea that incidence may not reach 1.0 was anticipated by Fracker (1936), who also introduced the concept of the multiple infection transformation, an equation relating incidence to severity. Agricultural epidemiologists have generalized K in nonlinear disease progress curves (Madden and Campbell 1990) and the examination of K has been applied to WPBR epidemics (McDonald and Dekker-Roberson 1998, McDonald and Hoff 2001). Fracker's (1936) ideas were combined with those of Bald (1970) to develop the concept of a factor that measures deviation in K over space for WPBR epidemics (McDonald and others1991, McDonald and others 1994).

If one assumes that total incidence can theoretically approach 1, then the difference between complete incidence and predicted asymptote, 1-K, may be attributed to lack of uniformity in distribution of resistance genes, microclimate,

or composition or density of inoculum. Predictions of K in different situations could be vitally important for breeding and deployment programs alike. For a breeding program, clumpy distribution of infection due to clumpy microclimate and/or basidiospore distribution can significantly increase the probability that rust-free candidate trees are susceptible escapes whose processing reduces screening efficiencies. However, asymptotes may also reflect effects of a major or minor gene for resistance, or situations in which even susceptible materials will persist on a site, and changes in K during an epidemic could indicate major changes in rust and/or pine populations (Kinloch and Byler 1981, McDonald and others 1994). Infection rates, especially comparative rates, are useful for assessing durable resistance (McDonald and Dekker-Robertson 1998).

Phenotypic Plasticity, Reaction Norms – Construction of Hypotheses, and Understanding G x E—Since a central tenet of breeding for resistance in any pathosystem is host/pathogen coevolution, it thus seems natural that geneticists and phytopathologists engaged in development, deployment, and maintenance of resistance should examine theoretical interpretations utilized by other disciplines to understand the implications of G x E in plant pathosystems. Indeed, evolutionary biologists have developed very powerful concepts and tools – those of phenotypic plasticity and reaction norms – not yet widely applied in phytopathology and sparingly used in forest genetics. Phenotypic plasticity is the ability of a genotype to express itself as different phenotypes in different environments. A few notable applications of this concept in forest genetics were found (Wu 1998, Wu and Hinckley 2001). As a basis for the following discussion, concepts from the excellent book by Pigliucci (2001) are liberally presented. Central elements in the concept of phenotypic plasticity are the reaction norm and its connection to analysis of variance as applied in quantitative genetics. Reaction norms are functions that relate the range of possible phenotypes a genotype can express across an environmental gradient. They are generally used to visualize genetic, environmental and G x E variance by comparing at least two genotypes, families (any relationship), populations, species, or groups of species across an environmental gradient. Any gradient of interest, e.g., light, temperature, moisture, nutrients, or host organisms, can be displayed. However, if a true gradient is not present, then sites or hosts must be compared in pairs or some kind of artificial gradient needs to be assumed. Reaction norms have a historic association with ANOVA as the preferred method of analysis. Summary statistics are often given in a suitable format such that all ANOVA elements are present, to wit: differences among across-environment means of the genotypes indicate genetic (G) variance; differences among across-genotype means of environments indicate environmental (E) variance; and when G and E interact in ways not predicted by the combined influence of across-environment and across-genotype means, there is plasticity (P) variance. Further note that if means for an individual genotype differ significantly across-environments, that genotype is plastic. This allows computation of the log-odds ratio and its associated standard error for hypothesis testing. A newly published book presents a multivariate approach to the analysis of G x E in a crop-breeding context (Yan and Kang 2003). Although examples of most types of organisms are presented in Pigliucci's (2001) book, fungi and plant pathosystems are notably absent, arguably because phytopathologists and mycologists have been slow to embrace the concepts and methodology of phenotypic plasticity and reaction norms. Yan and Kang (2003) devote two chapters to the analysis of pest resistance G x E in the context of crop resistance breeding rather than in terms used by evolutionary biologists.

To foster a dialogue about these and other questions, we will look at some blister rust pathosystem processes using the concepts and tools just outlined. We will apply the concepts of K, infection rate, inflection points, and reaction norms in a critical assessment of published data as well as updated data obtained from established plantings. Our intent is to advance the idea that becoming better rust detectives will lead to better development, deployment, and management of resistance genes in western North American 5-needle pines.

Materials and Methods

Data

To illustrate the utility of the reaction norm approach and in keeping with the disease detective approach to the study of WPBR epidemiology, reaction norms were constructed for seven cases from previously collected or published data. Case one examines time from inoculation to pustule development for inoculum sources from different geographic areas inoculated onto individual *Ribes* clones; data was obtained from a study (McDonald 2000) that was previously published using average responses over all clones. Reaction norms (fig. 1-3) were selected to provide an elementary lesson in recognizing types of trends that may be encountered. The second case identifies variation in three types of pine response to the Champion Mine race of *C. ribicola* obtained from different alternate hosts through a reexamination (fig. 4-5) of data from McDonald and others (1984). Case three demonstrates the use of reaction norms (fig. 6-7) to dissect effects of developmental stages, and possibly ontogenic resistance, using developmental reaction norms defined by periods of exposure to rust for WWP data published by Bingham (1966 and 1972). Case four uses reaction norms to demonstrate a classic G x E interaction (fig. 8) for WWP stocks grown at two *ex situ* locations in Japan (Yakota 1983). Case five demonstrates the fitting of incidence data from various classes of resistance phenotypes grown at two northern Idaho sites into disease progress curves (fig. 9); further examination of differences in reaction norm parameters among resistance and site categories are presented (fig. 10-12). Case six examines infection and disease progress parameters in three northern Idaho full-sib test plantation sites (Bingham and others 1973, McDonald and others 1994, McDonald and Dekker-Robertson 1998, Fins and others 2002); reaction norms are set up to compare parameters that were obtained for different resistance classes early versus late in the epidemic (fig. 13-16). Case 7 is a detailed examination of infection severity and incidence to reveal clues about the cause of unexpectedly high disease incidence on resistant stocks at one of the three sites.

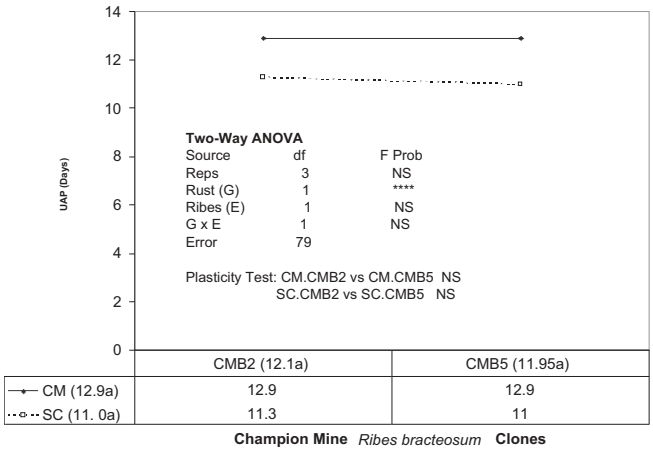


Figure 1—Reaction norm demonstrating significant genetic variance in number of days from inoculation to first appearance of urediniospores for two sources of *Cronartium ribicola* (CM = Champion Mine, OR; SC = Still Creek, OR) inoculated as aeciospores onto two clones of *Ribes bracteosum* collected at the Champion Mine site (Case 1 in text). ANOVA based on four replicated inoculations of 6 leaf disks for each *Ribes*-Rust combination.

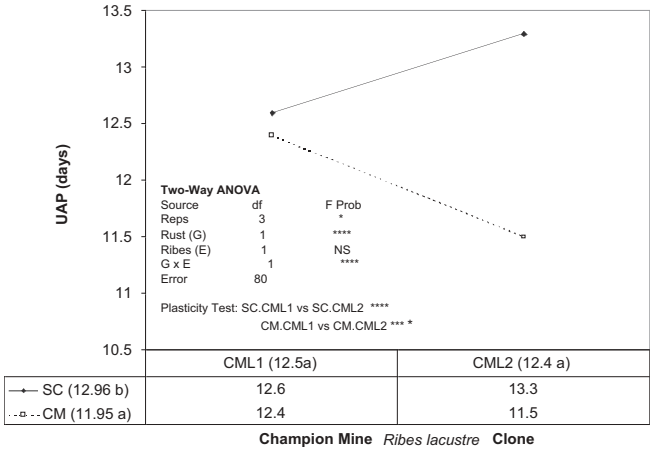


Figure 3—Reaction norm demonstrating significant genetic, environmental and G x E variance in number of days from inoculation to first appearance of urediniospores for two sources of *Cronartium ribicola* (CM = Champion Mine, OR; SC = Still Creek, OR) inoculated as aeciospores onto two clones of *Ribes lacustre* collected at the Champion Mine site (Case 1 in text). ANOVA based on four replicated inoculations of 6 leaf disks for each *Ribes*-Rust combination

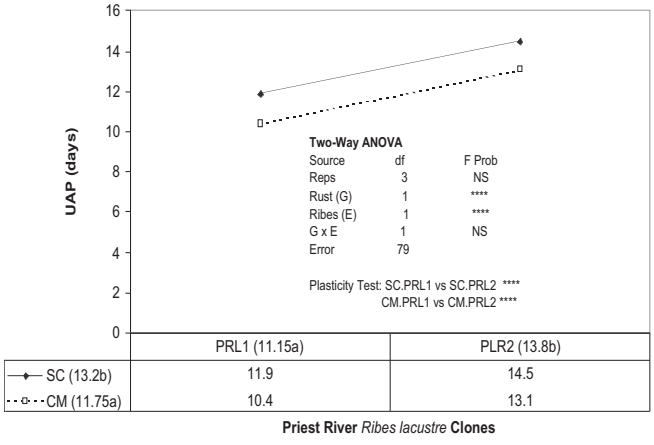


Figure 2—Reaction norm demonstrating significant genetic and environmental variance in number of days from inoculation to first appearance of urediniospores for two sources of *Cronartium ribicola* (CM = Champion Mine, OR; SC = Still Creek, OR) inoculated as aeciospores onto two clones of *Ribes lacustre* collected at the Priest River Experimental Forest, Idaho (Case 1 in text). ANOVA based on four replicated inoculations of 6 leaf disks for each *Ribes*-Rust combination.

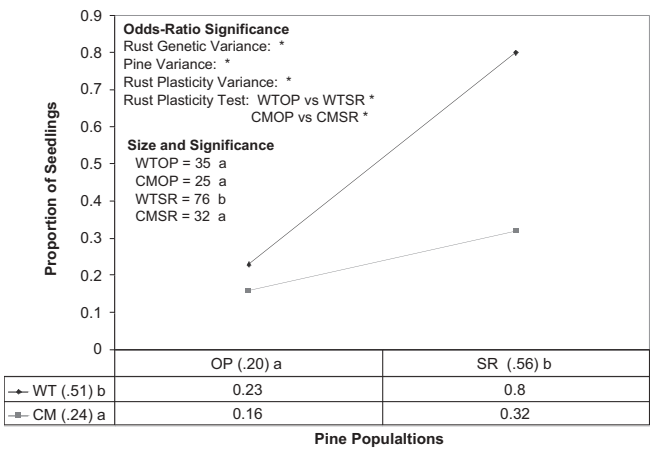


Figure 4—Incidence of premature needle shed resistance in *Pinus monticola* for two sources of *Cronartium ribicola* basidiospores (CM = Champion Mine, OR; WT = pooled Still Creek and Grass Creek, OR) inoculated onto 13 open pollinated pine families obtained from phenotypically resistant parents (OP) vs. seven full sib families obtained from Champion Mine and specifically selected for resistance to WT inoculum (SR) (Case 2 in text; data from McDonald and others 1984).

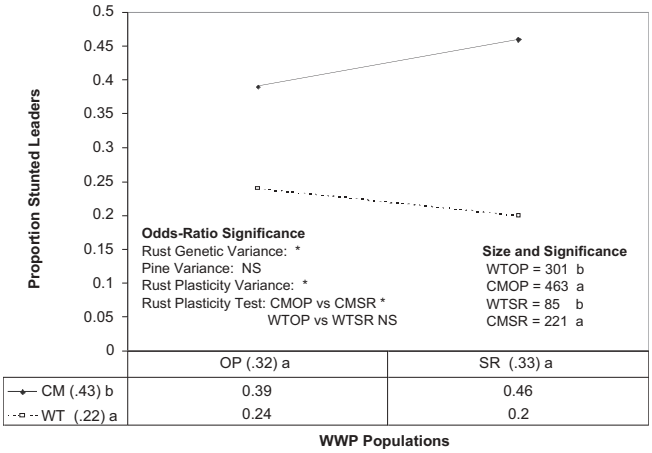


Figure 5—Proportion of stunted leaders in *Pinus monticola* for two sources of *Cronartium ribicola* basidiospores (CM = Champion Mine, OR; WT = pooled Still Creek and Grass Creek, OR) inoculated onto 13 open pollinated pine families obtained from phenotypically resistant parents (OP) vs. seven full sib families obtained from Champion Mine and selected for resistance to WT inoculum (SR) (Case 2 in text; data from McDonald and others 1984).

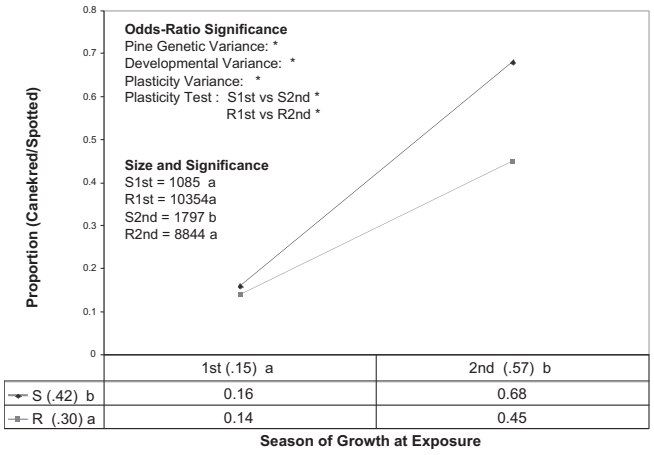


Figure 7—Effect of seedling development stage during rust exposure on stem infection in *Pinus monticola* (Case 3 in text; materials and definitions as in fig. 6).

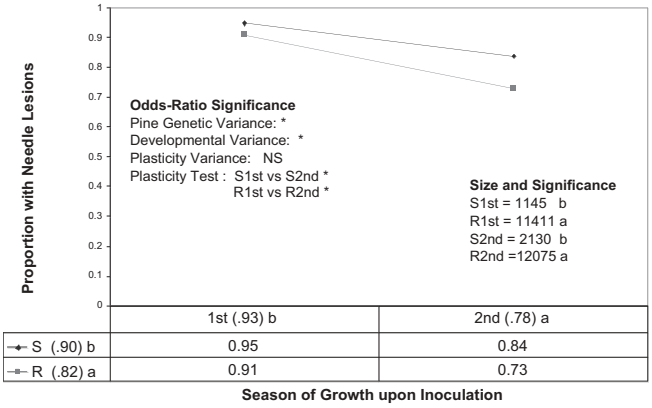


Figure 6—Effect of seedling development stage during rust exposure on needle infection in *Pinus monticola* (Case 3 in text): Incidence of infection for seedlings of phenotypically resistant parents (R; full-sib progeny) vs. susceptible infected parent trees (S; open pollinated progeny from high rust areas in northern Idaho) after exposure to a bulked source of *Cronartium ribicola* basidiospores from north Idaho at the end of first (1st) vs. second (2nd) growing seasons (Data from Bingham 1972).

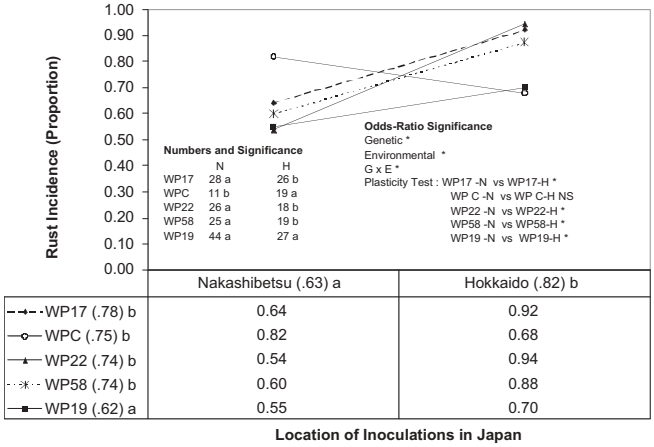


Figure 8—Genetic x Environment interaction affecting resistance in *Pinus monticola* (Case 4 in text): Incidence of canker infections for five half-sib families from crosses of north Idaho seed parents after inoculation with unique sources of *Cronartium ribicola* basidiospores at two localities, i.e. natural inoculations at Nakashibetsu, Japan and artificial inoculation at Hokkaido, Japan. (Data from Yokota 1983).

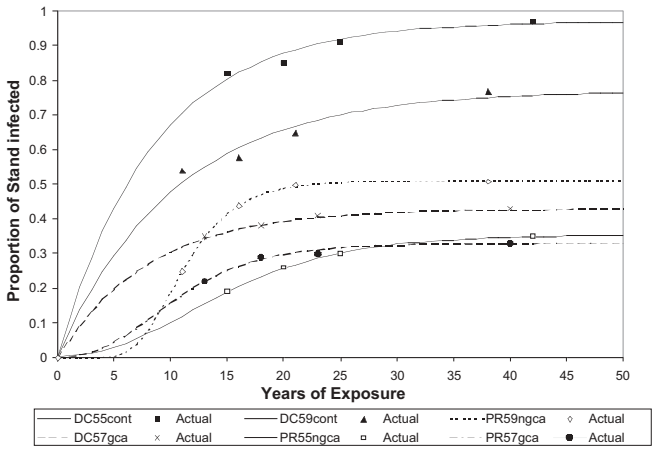


Figure 9—Disease progress curves for epidemics caused by *Cronartium ribicola* showing differences in disease incidence asymptotes (K) from fitting optimal nonlinear equations (Gompertz or monomolecular) to data from groups of *Pinus monticola* families representing three resistance pedigrees (GCA x GCA, NonGCA x GCA, and OP Controls) grown at Priest River (PR) and Deception Creek (DC) Experimental Forests located in northern Idaho, USA (Case 5 in text).

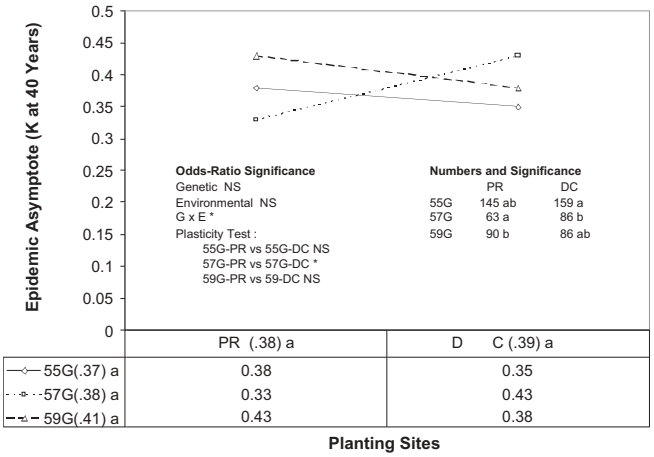


Figure 11—Disease incidence asymptotes (K) for *Cronartium ribicola* epidemics in groups of *Pinus monticola* full-sib families with a GCA x GCA pedigree planted in 1955 and 1956 (55G), 1957 (57G) and 1959 (59G) at sites in northern Idaho on the Priest River (PR) and Deception Creek (DC) Experimental Forests (Case 5 in text).

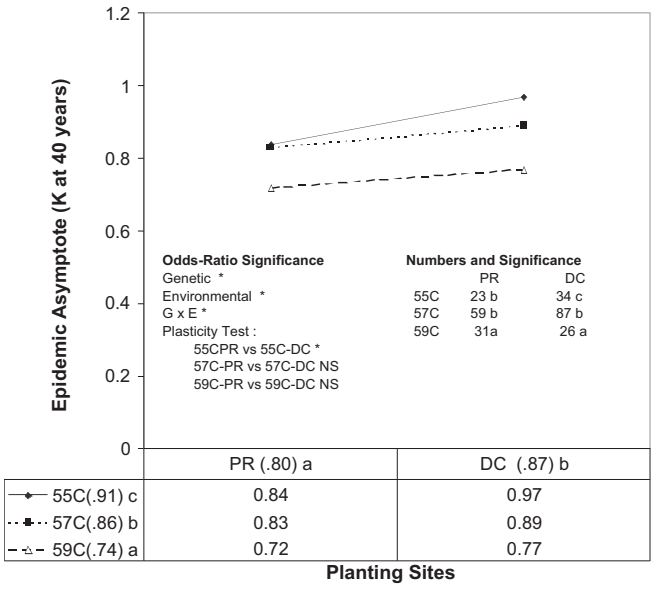


Figure 10—Disease incidence asymptotes (K) for *Cronartium ribicola* epidemics in *Pinus monticola* open pollinated control lots planted in 1955 and 1956 (55C), 1957 (57C) and 1959 (59C) at sites in northern Idaho on the Priest River (PR) and Deception Creek (DC) Experimental Forests (Case 5 in text).

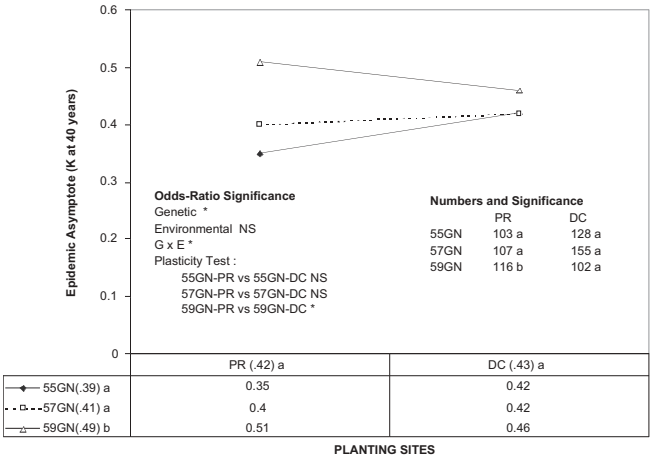


Figure 12—Disease incidence asymptotes (K) for *Cronartium ribicola* epidemics in groups of *Pinus monticola* full-sib families with a nonGCA x nonGCA pedigree planted in 1955 and 1956 (55GN), 1957 (57GN) and 1959 (59GN) at sites in northern Idaho on the Priest River (PR) and Deception Creek (DC) Experimental Forests (Case 5 in text).

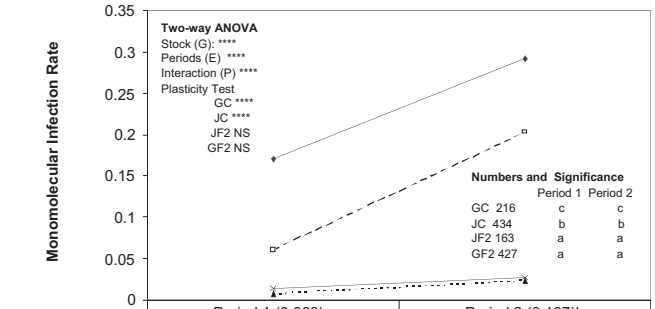


Figure 13—*Cronartium ribicola* infection incidence rate constants computed using the monomolecular equation for the first 11 years (period 1) vs. the last 14 years (period 2) during which infection was monitored in *Pinus monticola* control (Cont) vs. second generation resistant selections (F_2) planted at Gletty Creek (G) in northeastern Washington and at Jaype Mill (J) in northern Idaho (Case 6 in text).

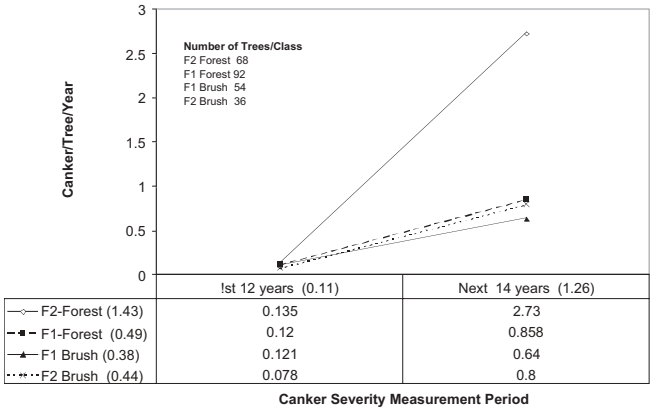


Figure 15—*Cronartium ribicola* average canker accumulation rates for first 11 years (period 1) vs. last 14 years (period 2) during which infection was monitored in *Pinus monticola* first (F_1) vs. second generation resistant selections (F_2) planted at Merry Creek in northern Idaho under forest vs. brush site conditions (Severity data supplemental to study described by McDonald and Dekker-Robertson 1998) (Case 7 in text).

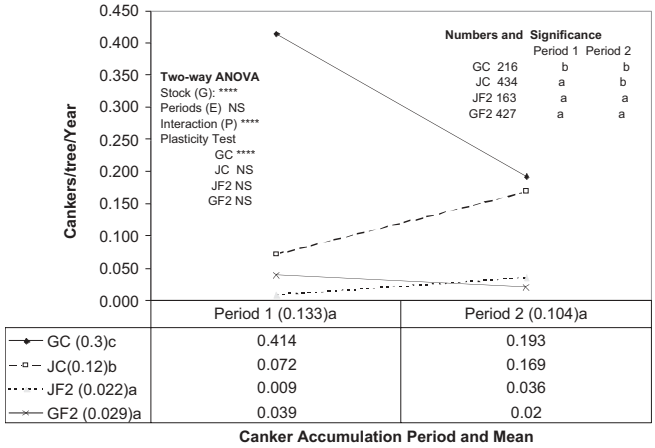


Figure 14—*Cronartium ribicola* average canker accumulation rates for first 11 years (period 1) vs. last 14 years (period 2) during which infection was monitored in *Pinus monticola* control (Cont) vs. second generation resistant selections (F_2) planted at Gletty Creek (G) in northeastern Washington and at Jaype Mill (J) in northern Idaho (Case 6 in text).

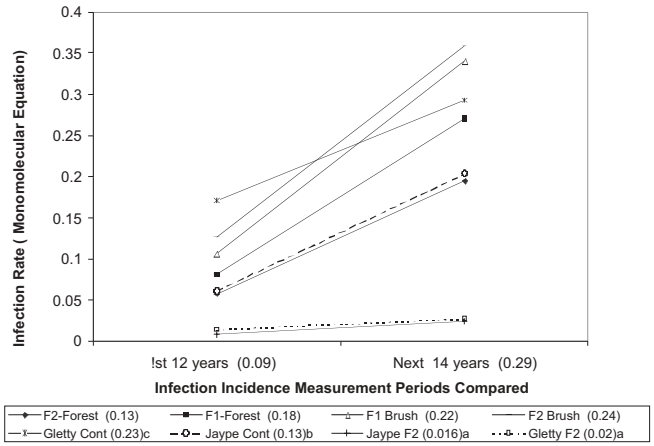


Figure 16—*Cronartium ribicola* infection incidence rate constants computed using the monomolecular equation for the first 11 years (period 1) vs. the last 14 years (period 2) during which infection was monitored in *Pinus monticola* control (C) vs. second generation resistant selections (F_2) planted at Gletty Creek (G) in northeastern Washington and at Jaype Mill (J) in northern Idaho vs. Merry Creek (Case 7 in text; based on data from McDonald and Dekker-Robertson 1998).

Statistical Methods

This paper utilized three main types of statistical tests: ANOVA with multiple means testing, log-odds ratios applied to means of proportions, and nonlinear curve fitting. Data were available for the *Ribes*-rust geographic variation experiment (McDonald 2000) to conduct standard analysis of variance (Systat 9.1 SPSS, Inc) for each set of four comparisons in the reaction norm format. These data meet the normal distribution assumption for ANOVA (see McDonald 2000). This format and details of reaction norm analysis can be found in Pigliucci (2001). Where multiple means were compared in pairwise format the Bonferroni correction was used (Systat 9.1). In the case of nonlinear curve fits, the standard error of the parameter estimate (disease asymptote) was computed by the curve fitting software (Table Curve 3.0 SPSS, Inc).

Analyses based on published data and new analysis of the vigor-quality plantings entailed comparisons of proportions; log-odds ratios were used to determine if any particular pair of proportions was significantly different (Sokal and Rohlf 1995). The standard error of the comparison was determined by taking the square root of the sum of the reciprocals of the number of observations in each cell of the comparison (Sokal and Rohlf 1995). To obtain 95 percent confidence limits, the standard error was doubled and then added and subtracted from the natural log of the odds ratio (Sokal and Rohlf 1995). When this range crossed zero, the proportions under comparison were considered not significantly different (Sokal and Rohlf 1995). Reaction norms require comparison of across-environment means of each family line or genotype, as well as comparison of each family x environment proportion. Mean proportion of all families in an environment must be compared across environments. To accomplish these comparisons, we computed each relevant mean proportion and estimated the standard error by computing the average number of observations per cell of the four cells associated with the proportions being compared.

Results and Discussion

Case 1: Uredinial Sorus Development Time

Data previously presented by McDonald (2000) but pooled over five *Ribes* clones were separated by clone x WPBR source and the period from inoculation to urediniospore appearance. The number of days until urediniospores appeared (urediniospore appearance period; UAP) was then subjected to a new ANOVA. Given that hosts of plant pathogens are a significant component of a pathogen's environment, our first example will include two WPBR populations (Champion Mine and Still Creek) compared on two *R. bracteosum* Douglas clones from the Champion Mine site (fig. 1) and two *R. lacustre* (Pers.) Poir. clones obtained from the Priest River site (fig. 2). We also include two Champion Mine *R. lacustre* clones (fig. 3). The important elements are the mean UAP's obtained from 24 leaf disks — four replications of inoculations of 6 disks each (McDonald 2000).

The across-environment means are important, as are the slopes of the reaction norms. Defining the "genetic" component as differences due to rust source, and "environment" as that due to rust clone in the first comparison (fig. 1), the across-environment means differ significantly, indicating significant genetic variance in the rust populations. There is no environmental variance (means for the two *Ribes* clones across rust populations do not differ), no plasticity variance (no G x E interaction), and no plasticity for either population (the individual environment means for each rust population do not differ). There would be no genetic variance if means for the two rusts were not significantly different. Next, we illustrate sloping parallel reaction norms and associated ANOVA (fig. 2). In this specific combination, the rust populations showed significant genetic variance (across-environment means differ), environmental variance (environmental means differ), and both rust populations show plasticity because means within a rust population differ across environments, but there is no plasticity variance (no G x E). Crossing or divergent reaction norms (fig. 3) also yield specific genetic information about *R. lacustre* clones CML1 and CML2: Genetic variance is present since the across-environment means differ, but environmental variance is not present since the mean performance of the two rust populations on *Ribes* clones, CML1 and CML2 do not differ. Variance for plasticity is present (significant G x E). If we were comparing rust clones derived from single spores, we could make the case that rust clones have heritable plasticity. Since we are comparing rust populations, we can only argue that the two populations are genetically different. Both populations are exhibiting plasticity, since their performances differ by *Ribes* clone.

The real power in such specific G x E comparisons resides in the fact that specific hypotheses can be created and tested. These hypotheses can then form the basis for designing specific experiments, which can include application of molecular techniques (Wu 1998, Pigliucci 2001). For example, figure 3 might suggest that genetic variation in *C. ribicola* is caused by allelic differences within mixed populations, variation in gene expression, or some other factor such as conditionally dispensable chromosomes or transposon activity. Conditionally dispensable chromosomes are small chromosomes that often contain genes coding for pathogencity that can be conditionally removed from the genome (Hatta and others 2002, Covert 1998). Transposons — transportable genetic elements — are known in the basidiomycotina (Fowler and Mitton 2000) and are known to influence the expression of host resistance genes in a rust pathosystem (Luck and others 1998). Another potential role for utilizing reaction norms for understanding pathosystems is to examine quantitative geographic and host differential responses beyond the qualitative virulent – avirulent dichotomy based on R genes, as discussed above.

Case 2: R Gene-Virulence Interaction

We will briefly discuss aspects of the CM (Champion Mine) race in regard to qualitative vs. quantitative differential reactions. The CM race was first described in 1984 (McDonald and others 1984) using a series of quantitative pine host responses relating mostly to reduction in frequency of expression of several resistance traits. Open pollinated families obtained from phenotypically resistant candidate trees growing in Oregon and Idaho were compared to a group

of full-sib families obtained from selection and breeding candidates obtained from the CM site (Kinloch and others 1999, McDonald and others 1984). Trees were inoculated with CM and wild-type inoculum and expression of various symptoms was recorded monthly for 24 months. Data were published (tables 4 and 5 in McDonald and others 1984) that now allow reaction norms to be developed for genetic interpretations. Responses of the OP and selected-resistant families with regard to premature needle-shed resistance (McDonald and Hoff 1970) yielded significant genetic and environmental (host genetic) variance when genotype is defined by host and environment is defined by kind of rust inoculum (fig. 4). Plasticity variance was indicated by the combination of nonsignificant (NS) and significant differences in the E means. The reaction norm interpretation is that both sources of blister rust carry genetic variation for plasticity since means from their cross-environment plasticity tests were significantly different. This analysis shows that the OP candidate families carried some premature needle shed resistance that was of equal effectiveness to both populations of the rust; i.e., they did not differentiate the rust populations. However, the resistant population selected by screening and breeding for resistance to inoculum collected at CM dropped from 80 percent expression of resistance to 32 percent. This is still an important level of resistance, which suggests some mechanism other than simple negation of an R gene may underlie susceptibility to rust variants capable of neutralizing R genes. An alternative explanation for the observed pattern in the R-gene-containing/R-gene-lacking couplet is variation in expression of R genes associated with host maternal cytoplasm (Kinloch and others 1999).

Before we leave the CM race, we should investigate the effect of this rust population on frequency of stunted leaders (McDonald and others 1984). Reaction norms were constructed (fig. 5) showing significantly greater incidence of stunted leaders by CM rust across host environments. There was no significant response of this trait to selection for resistance when inoculated with wild-type rust, but significant genetic and plasticity variances in the rust. The pattern of these norms indicates experimental conditions that could facilitate the study of physiological aspects of WPBR. This is especially interesting since the CM inoculum grown at the site of inoculation exhibited a significantly higher incidence of stunted leaders than did the two sources obtained at the CM site. In closing our discussion about CM rust, evidence of many kinds of differential interactions involving CM on both *Ribes* and pine hosts (fig. 1 to 5) sends a message that there is much more to host interactions with WPBR than simple virulence / avirulence alleles in the rust and R genes in the pine hosts. An initial step toward understanding these complexities should be to reanalyze the initial test (McDonald and others 1984) in light of the current knowledge about *Cr2* genes in WWP (Kinloch and others 1999).

Case 3: Ontogenic Resistance

In Bingham's (1972) large-scale screenings of 1966 and 1967, many thousands of seedlings in a number of control lots and full-sib tester families germinated one year late. Thus, seedlings belonging to the same seedlots were simultaneously inoculated at 1 and 2 years of age. Incidence of needle infection and subsequent incidence of cankers were analyzed for each seedling. For incidence of needle lesions, WWP clearly showed genetic variance, environmental (developmental) variance, and plasticity in both resistant and susceptible populations (fig. 6). No variation for plasticity was evident because both populations moved in the same direction. Thus, we can argue that age (primary needles vs. secondary needles) has an equal influence in both resistant and susceptible populations. This leads to the conclusion that screening efficiency could have been enhanced by early inoculation. As pointed out by Bingham (1972) the lower needle lesion incidence in older seedlings is surprising given that target increases about 4x by the end of the second season. Take note that almost 30,000 seedlings were included in this test (fig. 6).

Analysis of canker incidence clearly illustrates why primary needle inoculation did not work (fig. 7). Incidence of cankers does not differentiate populations under first season inoculation (within E1 NS). Meanwhile populations were clearly separable when secondary needles were inoculated. This result also indicates a large amount of resistance (spots but no cankers) in the control populations used in the 1964 and 1965 screenings. Bingham (1972) noted the essential differences from his perspective. So what, if anything, does the reaction norm perspective add? In this case, two obvious advantages are to focus attention on interactions and development of questions from "outside the box" by forcing consideration of a wider viewpoint. For example, comparative incidence of both needle and stem infections brings into question just how much and what kind of resistance was present in materials selected for "susceptible controls" in the 1950s after just 25 years exposure of the WWP to WPBR. During the Phase I screenings at Moscow, Idaho, incidence of combined infection (spots and cankers) varied from 0.5 to 1.0 for individual control lots, and no two inoculations utilized the same control lots (Bingham 1972). It appears that few of the WWP control lots supported cankering incidences > 0.99, as had five of eight control lots of EWP (Patton 1961).

Case 4: Analysis of Pine Rust G x E in Japan

The western breeding programs have been very aware of growth G x E and in some cases have gone to great lengths to capture its potential in selection programs (Kitzmiller and Stover 1996). In our introduction, we discussed indications gleaned from the literature that both rust-pine and rust-*Ribes* G x E may be of great import to understanding WPBR. We have just seen how reaction norms are constructed and interpreted, and from other sources (McDonald and Andrews 1981, McDonald 2000), that WPBR-*Ribes* G x E might play an important role in WPBR dynamics. Now we will take a closer look at WPBR-pine G x E.

Yokota (1983) provided sufficient data and descriptions of his experiments for us to apply a reaction norm analysis. Several full-sib F_1 families created within the north Idaho Phase I breeding program and two lots of susceptible north Idaho WWP were inoculated in Japan at two sites. Potted seedlings were transported from Hokkaido to Nakashibetsu 440 km eastward where they were exposed to basidiospores produced on local *Pedicularis resupinata* L. under natural

conditions. The stressful nature of the latter site is indicated by Yokota's statement that in a related test conducted the previous year, many WWP seedlings were killed by winter exposure to cold temperatures. In the experiment we are analyzing, seedlings were exposed from August 22 to October 16, 1978, and then transported back to Hokkaido. Identical families were exposed to artificial inoculation September 18 to 20, 1978, and again September 27 to 30, 1979, at Hokkaido to basidiospores that developed naturally on local *P. resupinata*. Mean spore cast for the two inoculations were 90 and 80/cm^2 (Yokota 1983). Both naturally and artificially inoculated seedlings were placed in the same nursery to allow development of symptoms. Needle lesions were not observed, but incidence of cankering was recorded based on continuous observations for 5 years (Yokota 1983). Reaction norms were constructed from Yokota's canker incidence data.

Our example is a bit light in numbers since the control at one site included only 11 seedlings, but features of the reaction norm facilitate the extraction of useful information. One might say that the differences noted resulted from different rust populations or inoculation procedures or both. At one site the seedlings received a two-month natural exposure, while at the other site they received two short-term artificial exposures that delivered only about 85 spores/cm^2. This is far short of the 2,000 spores/cm^2 or more that are delivered in artificial inoculations from *Ribes* leaves during screening and experimentation (McDonald and others 1984). The fact that one family group (WP22) went from having the lowest to the highest incidence upon change of location, while another went from highest to lowest (this change was not significant due to low numbers) argues that the comparison may have a message, despite the aforementioned shortcomings. For one thing, the families tested generally gave about 30 percent canker-free individuals after artificial inoculation in Idaho and in the Nakashibetsu test so that they still had 30 percent less incidence than controls (fig. 8). However, almost no resistance was expressed at Hokkaido (fig. 8). Significant differences were seen even though the trees spent only two months at Nakashibetsu. There were significant differences associated with family groups and lots of G x E expressed in families WP19 and WP 22. Is this complex picture caused by variation of virulence or aggressiveness on the part of the rust, or by environment acting on gene expression in the pine or the rust? We have seen that planting northern Rockies F_2 WWP at a low elevation (warm) coastal site increases susceptibility while planting the same material nearby at a higher and more northerly site resulted in expected performance (Hunt and Meagher 1989). If the Nakashibetsu site was consistently cold during initial colonization (as mentioned, WP seedlings died of cold exposure the previous winter) and the Hokkaido site was warmer during initial colonization, then cool temperatures during inoculation and early colonization is a common element that may be related to activation of resistance genes. Expression of resistance genes in some pathosystems is influenced by temperature (Pérez-Garcia and others 2001). Another possible source of G x E is needle physiology. In EWP, certain trees showing one-season needle retention also exhibited low WPBR canker incidence (Hirt 1944). Trees retaining one season's needles became more normal in their retention of three seasons of needles after transplantation to a new site, and there exhibited normal rates of infection (Hirt 1944).

Case 5: Vigor-Quality Western White Pine Plantations

Repeated measurements of experimental material [e.g., resistant material having or lacking general combining ability (GCA) as well as susceptible materials] growing in natural environments are a powerful source of information about the dynamics of WPBR epidemics. In 1955, 1957, and 1959, outplantings of early generational materials from the Phase I program (full-sib GCA x GCA and GCA x Non GCA, open pollinated GCA, and Non GCA) were made at three locations in north Idaho to monitor the vigor and quality of the resistant families (Steinhoff 1971, Goddard and others 1985). Control lots arising from seed collected from infected members of the same cohorts as the resistant selections were also planted. In 1953 and 1955, OP seed was collected from infected members of the cohorts of phenotypically resistant trees at the same five locations to serve as control lots for the 1957 and 1959 plantings respectively (tree location data on file). Controls for the 1955/1956 planting were collected, in 1951, from infected trees located outside of resistant-tree selection sites. Plantings were replicated at Priest River, Deception Creek Experimental Forest, and at Emerald Creek on the Saint Joe National Forest, all in northern Idaho, USA. Poor survival at Emerald Creek precluded further consideration of that site. Plantations were inspected for rust incidence in 1970, 1975, 1980, and 1997. The 38 to 42 year-old trees were inspected from the ground for canker incidence in 1997. Records of individual trees were checked for continuity across all dates and records of unknown mortality were removed. Any record of rust infection during the life of a tree placed it in the infected category. A few records lost continuity between the 1980 and 1997 inspections because specific trees could not be relocated. The 1980 data were published (Goddard and others 1985) and data for the other years are on file (Moscow Forestry Sciences Laboratory). Reaction norms were constructed using as data estimates of the asymptote (K) of canker incidence obtained by fitting nonlinear functions to rust incidence data recorded at about 13, 18, 23, and 40 years. Equations (Madden and Campbell 1990) fitting expectations of the monomolecular (monocyclic disease assumption), the logistic (polycyclic disease assumption with inflection assumed at 0.5 K), and Gompertz models (polycyclic disease assumption with variable inflection point) were calculated with Table Curve software (SPSS Inc). Each site initially received equal numbers of seedlings from the same families at each planting, although uneven numbers of losses due to planting occurred at each site. Each of the three years contained a unique mix of full-sib families for each resistance category. Pivotal to this discussion is the fact that the same mix of full-sib families was planted at the two sites for each planting time.

Estimated K and rate parameters of the best fitting equation, planting site, family groups, and standard errors of the estimated K and rate values are given in table 1. Out of nine combinations of site, family group, and planting year at Priest River, eight of the best fits (highest R^2 and lowest SE for K and infection rate) were derived using the Gompertz and, out of the nine at Deception Creek, eight were

Table 1—Fit of nonlinear equations to white pine blister rust canker incidence on full-sibs western white pine growing in the vigor-quality plantations located on northern Idaho Experimental Forest.

Full-sib group	Planting year	Planting location	Nonlinear[a] equation	K ± SE	Infection rate ± SE
OP Cont	1955	Priest River	Gomp	0.84 ± 0.025	0.16 ± 0.030
OP Cont	1957	Priest River	Mono	0.83 ± 0.017	0.08 ± 0.005
OP Cont	1959	Priest River	Gomp	0.72 ± 0.027	0.22 ± 0.058
GCA x GCA	1955	Priest River	Gomp	0.38 ± 0.018	0.10 ± 0.017
GCA x GCA	1957	Priest River	Gomp	0.33 ± 0.009	0.20 ± 0.046
GCA x GCA	1959	Priest River	Gomp	0.43 ± 0.011	0.25 ± 0.031
GCA x N	1955	Priest River	Gomp	0.35 ± 0.003	0.14 ± 0.007
GCA x N	1957	Priest River	Gomp	0.40 ± 0.009	0.18 ± 0.028
GCA x N	1959	Priest River	Gomp	0.51 ± 0.003	0.32 ± 0.011
OP Cont	1955	Deception	Mono	0.97 ± 0.024	0.12 ± 0.013
OP Cont	1957	Deception	Mono	0.89 ± 0.032	0.11 ± 0.015
OP Cont	1959	Deception	Mono	0.77 ± 0.046	0.10 ± 0.017
GCA x GCA	1955	Deception	Gomp	0.35 ± 0.008	0.14 ± 0.016
GCA x GCA	1957	Deception	Mono	0.43 ± 0.005	0.12 ± 0.007
GCA x GCA	1959	Deception	Mono	0.38 ± 0.015	0.11 ± 0.015
GCA x N	1955	Deception	Mono	0.42 ± 0.007	0.11 ± 0.008
GCA x N	1957	Deception	Mono	0.42 ± 0.018	0.12 ± 0.022
GCA x N	1959	Deception	Mono	0.46 ± 0.055	0.06 ± 0.016

[a]Gomp = Gompertz and Mono = Monomolecular equations (Madden and Campbell 1990).

derived using the monomolecular model. This indicates the rust was behaving in a fashion expected by a monocylic disease cycle at Deception Creek and in a polycyclic fashion at Priest River (fig. 9). The fit of these standard disease progress curves to the long-term WPBR canker incidence data was generally excellent for 18 combinations of families x site (table 1). Each genetic group seems to have its own unique path (fig. 9). However, some common patterns are evident. All attained their unique asymptote at about 25 years. At these sites and with these materials, once the plateau was reached, it has been stable. Although a stable K value at 25 years may not be obtained for epidemics at all sites, as demonstrated in Kinloch and Byler's (1981) long-term data collected at Happy Camp during the onset of the outbreak of the Happy Camp race in resistant SP, it is nonetheless true that when a stable predicted K value is obtainable, it can be an excellent trait for reaction norm analysis. If our plantings were composed of clones, or even full-sib families, instead of groups of full-sib families, we would have some very robust genetic information for drawing hypotheses about G x E interactions, as has already been demonstrated with poplars (Wu 1998, Wu and Hinckley 2001).

In these plantings, significant differences in K parameter could be due to clumpy distribution of basidiospores, clumpy microclimate, or variation in resistance among trees within a resistance category (McDonald and Hoff 2001). *Ribes* were eradicated from the vicinity of the plantations prior to establishment (Steinhoff 1972), and few bushes can be found in the local vicinity today. If a susceptible control lot can be shown to approach an incidence of one, then we can assume that departures from unity are due to resistance (McDonald and Dekker-Robertson 1998). When K is not constant throughout an epidemic, rust incidence alone is an inadequate measure of disease behavior. Under such cases, considering canker counts may be required to augment the use of incidence data; a multiple infection transformation function can then be used to generate predictability and for understanding irregular rust behavior.

We begin our analysis of the vigor quality (VQ) plantings by inspecting the reaction norms of the susceptible controls (fig. 10). Rust incidence of the 1955 OP controls at Deception Creek reached 0.97 after 42 years of exposure (fig. 10). Even through the 1955 control lots exhibited significantly lower rust incidence at Priest River than at Deception Creek (fig. 10) we will assume that potential K = 1 at both sites. The significant across-environment means for all three populations [significance estimated by log-odds ratio 95 percent confidence limit according to Sokal and Rohlf (1995)] is taken as evidence of a genetic difference among the OP seedlots. This difference presumably arose from changes in pollen cloud during the 4 years (1951 to 1955) over which the OP seedlots were being collected. Further, the significant difference between the within-environment K means suggests either that expression of the putative accumulated resistance was affected by the environment or that the rust populations differ. The significant interaction among 1955 vs. 1959 controls across environments argues in favor of heritable variation in plasticity of the pine but against variation in virulence among these rust populations.

The full-sib GCA x GCA analysis was similar to the GCA x nonGCA resistance in that E was not significant and P was significant. These groups of families exhibited a nonsignificant G variance. Nevertheless, the downward slopes between the 1955 and 1959 plantings indicate the potential for a significant G (fig. 11). Full-sib crosses between GCA and other parents (mostly nonGCA) exhibited variable behavior across the sites (fig. 12) that are probably due to differential expression of genes. The families included in the 1955 and 1957 plantings show no distinctions at either site (fig. 12). The site-mean Ks do not differ (no E variance) but the K for the 1959 planting at PR is high and performance of the 1959 planting at PR and DC is significantly different, leading to significant G and P variances. The absence of a difference among sites for two family groups argues for similar rust at both sites.

An over-all conclusion from this analysis of K based on evidence of an overall reduction of the asymptote of disease incidence at the end of the epidemic of 0.16 in the OP controls in just 4 years (fig. 10) is that the WWP population in north Idaho may have the natural capacity to respond very quickly to rust pressure. Possible explanations are rapid and significant changes in frequency of resistant genes or expression of induced resistance that could even be partially transgenerational in areas of extreme WPBR impact. However, we also need to remember that the WPBR pathosystem is composed of other factors, such as epiparasites, that may have been subject to change, so that some alternative sort of disease attenuation or damping of the ability of *C. ribicola* to damage pine might be in play (Pfennig 2001, Ebert 1998, Levin and Bull 1994, Roy and Kirchner 2000).

Long-term performance of the vigor-quality plantations also indicates that overall early resistant populations obtained from the Phase I program performed equally well at both sites in that both kinds of crosses resulted in about 60 percent clean trees. Individual groups of families showed significant G x E for expression of resistance as well as differences among the groups. These differences are more than likely traceable to individual full-sib families that made up the groups. Results from the plantings also strongly indicate that perhaps site is more important than genetic background in determining whether local epidemics will behave in characteristically monocylic or polycyclic fashions. An explanation for the existence of two kinds of epidemics would add much insight to our understanding of rust epidemics. It is important to remember that well-characterized materials (full-sibs given 50 years of natural rust exposure and subjected to frequent examinations for rust behavior) are an experimental and developmental treasure that we should make every effort to protect and continue to develop.

Case 6: Changes in Rate "Constants" at Full-Sib Resistance Evaluation Plantations in Northern Idaho

One of the final tasks associated with completing the Phase I program was establishment of three test plantations: Merry Creek (MC), Gletty Creek (GLC) and Jaype Mill (JM) (Bingham and others 1973, McDonald and others 1994, McDonald and Dekker-Robertson 1998, Fins and others 2002). MC and GLC are replicate sites, each consisting of 36, 0.4 ha plantings laid out in a randomized complete block design for four stock types (Bingham and others1973). Each contains full-sib F_2, F_1, B_1 (backcross from F_1 to original parents), and local OP controls (that is, different seedlots representing different genetic backgrounds). MC, established in 1970, is located about 82 km east of Moscow, Idaho. GLC, established in 1972, is about 205 km northwest of Moscow near the town of Newport, Washington. JM, established in 1971, is about 125 km southeast of Moscow and contains only control and full-sib F_2 stock. At MC, a 225-tree subset of permanent sample trees from each resident stock had been selected, tagged, and inspected for rust incidence and severity at 2, 4, 6, 12, and 26 years. Many of the original 225 selected plants at MC have since been lost to animals and other unknown causes to the extent that the original design was destroyed (McDonald and Dekker-Robertson 1998). A hot site-preparation burn that stimulated a heavy stand of evergreen ceanothus (*Ceanothus velutinus* Douglas) further compromised the design. In response, the stand was divided for analysis into two site classes: brush and forest. This partition produced two classes of about equal size for all stock types, as described in an earlier report (McDonald and Dekker-Robertson 1998). The GLC and JM plantations remained largely intact, but each experienced lower rust incidence than MC and were inspected only 3 times (1973, 1983, and 1996). Incidence in 1973 was too low for analysis. The JM layout paired F_2 and control plots having 64 planting spots. In each plot, WWP seedlings alternated with those of grand fir (*Abies grandis* (Dougl.) Lindl.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.). All 32 WWP on each plot were inspected each time. Blocking at these plantations enabled use of ANOVA to construct reaction norms of infection rate and canker accumulation rate for two periods: plantation establishment to 1982, and 1982 to 1996. Infection rate was computed using Madden and Campbell's (1990) equation 18 – the monomolecular equation where K is assumed to equal one and initial incidence is set to zero. Rate of infection from 1982 to 1996 was computed using the same equation with initial incidence set equal to the 1982 value. Canker accumulation rate was initially computed by dividing the average number of cankers in 1982 by the number of years of exposure, but for the final period, the number of cankers in 1982 was subtracted from the 1996 number and the result divided by 14 years. These values were subjected to ANOVA (Systat 9.1, SPSS) and reaction norms constructed to compare stocks growing at JM and GLC.

All variances for comparison of WPBR annual infection rate between the two periods of assessment were highly significant. The across-environment mean indicated that the local GLC control was almost 2 times more susceptible than the Jaype local control growing at Jaype, yet examination of F_2 material that was of the same genetic background at both sites indicated that the sites were of equal hazard (fig. 13). Could an explanation be found in natural selection operating at different levels of selection in different natural stands? The expectation is that WPBR had a much larger impact on WWP near the JM site on the Clearwater National Forest than in the GLC vicinity on the Colville National Forest. The significant interaction term is also of interest. The controls show no differential interaction as materials at

both sites showed a proportional gain in infection rate for the second period. The interaction is generated by the nonsignificant plasticity test for the F_2 at both sites (fig. 13).

Cankering rate provides a different and unexpected result (fig. 14). In contrast with the increasing parallel reaction norms for infection rate in the controls over the two periods (fig. 13), cankering rate moved from a difference of 6x to a nonsignificant difference. While values were much smaller and differences not significant, the trend for the F_2 was the same. In addition, the JM cankering rate appeared flat and was not significantly different between periods. The GLC control illustrated that infection rate can increase while cankering rate is simultaneously decreasing. Does the JM control represent a pathosystem that is coming to a stable asymptote and therefore a stable and predicable K parameter? On the other hand, could the increasing cankering rate coupled with an increasing infection rate signify an impending unstable K?

Case 7: Anatomy of an Unexpected Epidemic—The Merry Creek Plantation

An expected epidemic at the MC site provides some insight into stability or instability of K, although the relatively small numbers of designated "sample" trees remaining from plantation establishment to the end of sampling removed any chance of computing significances in the standard fashion. The good news is that enough of the sampled population remained in records and on the ground to establish patterns and generate hypotheses. Infection rate and cankering rate were computed as for GLC except that the F_1 was used as a substitute control because none of the highly susceptible "true" control seedlings survived to the second period (fig. 15 and 16). All resistant stocks exhibited nearly equal infection rates during the first period when all the controls were becoming infected and dying (fig. 16) (McDonald and Dekker-Robertson 1998). Most infection rates increased 7 to 8x with very little differentiation between the brush and forest sites for the F_1 (fig. 16). In striking contrast, the F_2 stock growing in the forest site-type had rates of canker incidence increase by 20x (fig. 15). Infection rate at MC shows interesting patterns when displayed with the F_2 data from JM and GLC in a common format (fig. 16). Of note is the almost parallel nature of most reaction norms. The MC forest-site-type F_2 is most similar to the F_2 growing on the low hazard GLC and JM sites and is not distinguishable from the JM control in spite of the huge increase in cankering rate. This argues that the entire increase in number of cankers occurred on already infected trees! How can such an increase in amount of inoculum not result in an increase in number of previously clean trees becoming infected? An additional observation is that the highest overall rate increase (F_2 brush-site-type) was closely followed by the F_1 brush-site-type. Did the presumed large increase in aeciospore production by F_2 forest-site-type trees result in increased infection rate on F_1 and F_2-brush trees? Allowing ourselves to consider even "outside the box" possibilities, could this pattern signify spontaneous local appearance of a microcyclic form of WPBR capable of infecting pine to pine, and if so, might it recently have evolved, or might it be a new arrival? WPBR is known to encompass microcyclic forms or species (Imazu and others 1991, Imazu and Kakishima 1995). An intercontinental "river of dust" has been demonstrated to allow viable transport of spores of some fungi (Griffin and others 2002); could microcyclic aeciospores from Japan occasionally have survived such a journey?

These relationships can be studied in other ways. It has been argued (McDonald and Dekker-Robertson 1998) that the increase in infection at MC above the expected K of 0.34 in the F_2 was just the continuation of a constant rate that after 26 years resulted in the observed 0.89 incidence of infection. Since 7 out of 26 plantings of F_2 stock in north Idaho have exceeded the expected asymptote of 0.34 (McDonald and others 1994, Fins and others 2002) in the 12 years or less it took MC F_2 to exceed the threshold, a definitive answer about the causal dynamics is demanded. As a focal point for this discussion (not intended to convey statistical significance), we present a hypothetical rust progress curve for the MC F_2 based on some of the tools we have been discussing (fig. 17). By fitting the monomolecular equation (McDonald and Dekker-Robertson 1998 equation 1) using the first four data points from MC (Table Curve 2D, SPSS), a K of 0.5 is obtained. If we next assume the appearance of a variant that can overcome the resistance that was responsible for the K = 0.5 of the first 10 years of the epidemic and then simulate the behavior based on a new timeline, with the 10th year as year 0, then incidence in year 10 = 0, 12^{th} = .5 and 26^{th} year = .89. The perfect fit with K = .89 that these three points allow (fig. 17), illustrate the limitations of fitting a multiparameter function to a small data set but is also intriguing. This figure illustrates some important points. Control and F_2 stock are clearly delineated and the hypothetical curve is similar to the actual curve shown for SP at the Happy Camp site (Kinloch and Byler 1981). Interruption of the established cycle of inspections at MC by a bureaucratic decision to stop WPBR research in northern Rocky Mountains in 1983 precluded knowing the exact shape of

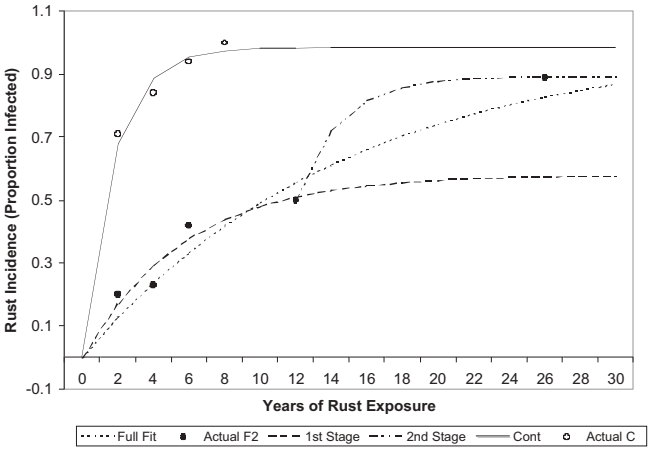


Figure 17—Expected rust progress curve for Merry Creek site (north Idaho) second generation resistant families (F_2) if epidemic asymptote were breached by *Cronartium ribicola* adjustment (plasticity or evolution?) compared with control lot. Both populations growing on forest site-type (Case 7 in text; data from McDonald and Dekker-Robertson 1998).

the MC progress curve. However, the hypothesized hump in the rust progress curve (fig. 17) might even now be verified or rejected by aging cankers at MC after the fact, in order to construct a reasonable progress curve.

Summary and Conclusions

The above discussion reinforces several significant lessons relating to resistance breeding, deployment, and management of all western five-needle pines. Of primary importance, pathosystems of long-lived plants demand long-term commitment in an atmosphere that fosters freethinking as well as commitment and focus to getting a big job accomplished efficiently. This probably means that lasting and workable relationships need to be forged between research and the practical breeding programs. Efforts in the past (Kinloch and Byler 1981, McDonald and others 1984, McDonald and others 1991, Kinloch and others 1999) exemplify such cooperation and sharing of data, and should be encouraged. The breeding programs generate large amounts of reliable data that are seldom subjected to peer review but could be of inestimable value, provided that such programs ensure that uniform and "adequate" control crosses are routinely included in artificial inoculations. Greater interregional cooperation may also help elucidate new resistance mechanism, for example mechanism "X" that USDA Forest Service Region 6's breeding and screening program at Dorena has observed in northern Cascade Mountain populations and that could potentially be related to resistance mechanisms in the northern Idaho WWP populations. Examination of this mechanism could be of critical importance in delineating seed zones and understanding the genetic structure of host populations. Recent studies of migration patterns of whitebark pine strongly suggest a north-south dichotomy whose boundary is in the southern Washington Cascades (Richardson and others 2002). This boundary could also be characterized by a significant change in the kinds and numbers of resistance genes in both WBP and WWP. Tools that are presently available to assess field performance are powerful, useful, and relatively inexpensive, if appropriate experimental designs and inspection intervals are maintained. Parting guidelines are as follows: Establish permanent plots so that adequate numbers of individuals can be tracked for at least 50 years. Ensure that repeated and blocked designs will provide sufficient numbers over time. Define measurement protocols to ensure continuity of data quality through changes in personnel. Ensure that canker incidence and severity, age, and size are recorded for whole trees to provide data appropriate for epidemiological analysis. Embrace the potential discriminatory power of pathosystem G x E by ensuring that genetic groupings to be tested (clones, full-sib families, half sib families and etc) are replicated in at least two environments. Finally, and perhaps most importantly, create customized control lots having known behavioral specifications and use them to link plantings in different geographic regions.

When dealing with long-term pathosystems, refrain from thinking "inside the box". For example, a strong correlation had been expected between density of *Ribes* populations and resulting WBBP impact (McDonald and Hoff 2001). Quite to the contrary, evidence is accumulating that such correlations are weak in the northern Rockies (Toko and others 1967, McDonald unpublished data) and southwestern Oregon (McDonald unpublished data). Two explanations are evident. First, western basidiospores may be more robust or travel further than assumed so that far fewer telial infections are needed to cause a corresponding amount of pine damage expected under the 300m "limit" of basidiospore spread. Second, other hosts could be involved; forms of *C. ribicola* are known in Japan and South Korea that alternate to *Ribes* spp and *Pedicularis*; in Japan and Germany, a form cycles to *Ribes* only; in South Korea, a form cycles only to *Pedicularis*; and in Canada it cycles to *Ribes* (Stephan and Hyun 1983). There is one report of a single branch of one plant of common red paintbrush (*Castillia miniata* Douglas, a North American Scropulariaceae) inoculated with WPBR that produced teliospores (Hiratsuka and Maruyama, 1976), and another report of artificial infection of this host but without spore production (Patton and Spear 1989). However, infections of artificially inoculated plants of this and other Scrophulariaceae were not obtained at multiple field sites by Hunt (1984), despite successful infection of "appropriate" *Castilleja* hosts after inoculation with stalactiform rust. Another potential problem with measuring WPBR susceptibility is the assumption that any old woods run collection of the pine host will make an adequate control. Custom controls should be developed and maintained for use by all breeding programs. This paper presented initial evidence that unknown mechanisms may cause rapid accumulation of "resistant" WPP. Theoretical discussions about disease attenuation are beginning to appear that invoke various kinds of evolutionary and plasticity (polymorphisms and polyphenisms) adjustments (Pfennig 2001, Ebert 1998, Levin and Bull 1994, Roy and Kirchner 2000). If the WPBR pathosystem is as dynamic as we have suggested, then an entirely new plan of attack may be needed to ensure that we can successfully restore and/or maintain ecosystems containing or requiring a five-needle pine component.

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