Whitebark Pine Germination, Rust Resistance, and Cold Hardiness Among Seed Sources in the Inland Northwest: Planting Strategies for Restoration

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Abstract: A synthesis of several studies highlights above-average performing seed sources (n = 108) of whitebark pine (*Pinus albicaulis*), which practitioners can utilize for restoration, wildlife habitat improvement, and operational planting programs. It is the first report of this magnitude of blister rust resistance for this species. Whitebark pine does have genetic variation and demonstrated resistance to white pine blister rust, increasing from the southeast to the northwest in the Inland Northwest. Early outplanting reports have shown that some seedlings have frost damage or exhibit increased mortality in cold pockets or swales. Cold hardiness, measured in late winter on a smaller sample of sources (n = 55), also showed genetic variability increasing from the northwest to the southeast. Seed zones were delineated by Mahalovich and Hoff (2000) based on information on relative rust hazard and demarcation of mountain ranges. These geographic seed zones support conservative seed transfer with a special emphasis on blister rust infection levels. Sufficient variability exists to maintain these seed zone boundaries, because whitebark pine exhibits more of an intermediate adaptive strategy as compared to the generalist adaptive strategy of western white pine (*P. monticola*). Based on this composite information, it is feasible to outplant whitebark pine without the additional delay of waiting until blister rust resistant seedlings are developed from a breeding program. There are sources within each seed zone that have both rust resistance and greater cold hardiness, so those factors should not limit tree planting for restoration or critical wildlife habitat improvement objectives.

Typical stock orders involve container-grown seedlings. A comparison between Economy and copper-lined Ray Leach Super Cell Cone-tainers™ (10 in^3 [164 cm^3]) shows no advantage to using copper lining.

Keywords: *Pinus albicaulis*, progeny test, genecology, heritability, electrolyte leakage test, index of injury

Introduction

Whitebark pine (*Pinus albicaulis*) plays a vital role as a keystone species in upper subalpine ecosystems, likely determining the ability of large numbers of other species to persist in the community (Primack 1998). Whitebark pine is a food source for grizzly bears, Clark's nutcrackers, and red squirrels, and is a foundation species for watershed protection by regulating runoff and reducing soil erosion. It is a species that quickly becomes established as a pioneer species following disturbance. Seedlings are very hardy and tolerate drought more readily than other conifers.

The number of acres in whitebark pine is rapidly dwindling (Scott and McCaughey 2006). High infection levels of white pine blister rust (*Cronartium ribicola*) are causing extensive mortality, with a secondary impact of losses in cone production whenever reproductively mature trees are infected and killed. Epidemic infections of mountain pine beetle (*Dendroctonus ponderosae*), selection against a pioneer species by fire suppression, and catastrophic wildfire are also causing extensive mortality. Whitebark pine is susceptible to cone (*Conophothorus* spp., *Dioryctria* spp., *Eucosma* spp.) and seed insects (*Megastigmus* spp.), seed-borne fungal diseases (*Sirococcus strobilinus*, *Calocypha fulgens*), and damping-off in seeds and germinants (*Fusarium* spp.). Once sufficient cone production is absent or curtailed, the primary dispersing agent, Clark's nutcracker (*Nucifraga columbiana*), moves onto other species like ponderosa pine (*P. ponderosa*) or other locations with the possibility of not re-colonizing the impacted area at a later time. Successful natural regeneration is not keeping pace with mortality. Ironically, the reasons for dwindling acres of whitebark pine identified above are also the same factors that make natural regeneration a questionable tool for restoration.

Rationale commonly cited for not proceeding with outplanting include: whitebark pine is not a commercial tree species; blister rust resistant seedlings are not available (this paper will show they now are); and costs of producing a seedling are perceived to be too high. Container seedling costs in northern Idaho range from U.S. $0.75 to 3.00 per seedling depending on nursery, container type, and seedling age (Burr 2005; Klinke 2005).

Whitebark pine may have one of the highest susceptibilities to blister rust of any of the five-needle pines (Bingham 1972; Hoff and others 1980), but individuals express notable resistance. An effective restoration program involves identifying and developing blister rust resistance. To accomplish that objective, patterns of genetic variation in a group of key adaptive traits need to be known, as well as their relationships to each other (genetic correlations) and how heritable they are. The strength and repeatability of each trait determines the restoration strategy for each species. Rust resistance in whitebark pine is a two-pronged strategy patterned after western white pine (Mahalovich and Dickerson 2004; Mahalovich 2005). First, families exhibiting resistance following an artificial inoculation with blister rust are selected based on an index score. Then individuals within superior families are selected for additional rust resistance, cold hardiness, and height performance. This is the first reported rust screening of this magnitude in whitebark pine.

The key to all of these traits is a focus on rust resistance (the ability to survive repeated infections), rather than the complete absence of infection (immunity), which would apply undue selection pressure on the rust, placing the host species at a continued disadvantage over time. Only one trait out of the seven evaluated typifies an immunity response (no-spot) (table 1).

Whitebark pine is hardy and drought tolerant; however, germinants and seedlings are stressed in frost pockets and cold swales (Scott and McCaughey 2006). In general, climatic races become adapted to particular environments as a result of natural selection. Typically, sources from milder climates often are not sufficiently cold-hardy when moved northward or when lower elevation sources are moved up in elevation. The practical implication of cold hardiness is also critical in restoration efforts in addition to rust resistance. Physiological testing is a means to determine the condition of nursery stock and to predict how it will respond to treatment or end use. The electrolyte leakage test can be used to measure cold hardiness and detect tissue damage. The principle of this test is that when cell membranes are damaged, electrolytes leak out into the water in which the tissue is immersed and can be measured by the conductivity of the solution. The test for damage is nonspecific; but in the case of cold hardiness, the damaging agent is known because the tissue is frozen. The 50 percent index of injury is used as the benchmark for cold hardiness because it is usually the midpoint on the regression curve of temperature versus injury and has the smallest confidence interval (Tinus 2002).

Materials and Methods

Stratification, Sowing, and Growing of Test Seedlings

Seeds for the test were sown in 1999 at USDA Forest Service Coeur d'Alene Nursery, Coeur d'Alene, Idaho from cone collections representing the geographical range of the species in the northern Rocky Mountains. Selected seedlots span 5° in latitude, 9° in longitude, and 1,900 to 3,300 m (6,235 to 10,825 ft) in elevation. Whitebark pine is a wind-pollinated species. These open-pollinated, individual-tree cone collections are assumed to be genetically representative of the area in which they were collected and are hereafter referred to as seed sources. The target number of sources for the study was a minimum of 100; 115 had an adequate number of seeds to proceed with sowing. During this timeframe, a large operational cone collection was made on the Shoshone National Forest (Wyoming). Requests to sow and plant from this seedlot throughout the northern Rockies were being made without information on whether the collection was rust resistant. As a result, sufficient numbers of container seedlings were reserved from general nursery operations to be included in the rust inoculation and testing phase of this study. Examination of the origin data for all seedlots suggests 54 unique areas are represented overall.

For seed coat disease control prior to stratification, seeds were soaked in a bleach solution of one part 6 percent sodium hypochlorite to two parts water for 10 minutes. Seeds were then rinsed four times in fresh water, placed in mesh bags, and soaked in cold running tap water for 48 hours. After a 28-day warm stratification period at 20 °C (68 °F) and a 1-hour running water soak, the mesh bags of seeds were placed in new 1-ml plastic bags and placed in a dark stratification room at 2 °C (36 °F) for 60 days. The weekly running water soaks were continued during this cold stratification as described by Burr and others (2001).

At the completion of cold stratification, seeds were not nicked with a scalpel to overcome seed coat dormancy, but instead were sown directly into the sphagnum peat-Douglas-fir wood chip blended growing medium in January 1999 in Ray Leach Super Cell Cone-tainers™ (Super Cells) (10 in^3 [164 cm^3]). A smaller sample of these Super Cells had copper lining to evaluate differences between the Economy and copper-lined containers. The growing environment was monitored and controlled with a computer integrated system. Heat was applied as needed with gas forced-air heaters, with heat tubes situated under benches. Photoperiod extension was accomplished with sodium vapor and metal halide lamps.

Table 1—Description of blister rust resistance traits, mechanisms, and selection strategies used in whitebark pine in the USDA Forest Service Northern Region.

Trait Name	Description	Selection strategy	Traits used to determine index score	Mean[a]	Standard deviation
Needle lesion frequency (NLF)	Reduced number of needle spots	Family Selection	X	0.36	0.58
Early stem symptoms (ESS)	Reduced number of early stem symptoms (cankers)	Family Selection	X	0.07	0.10
Bark Reactions (BR)	Increased number of callus formation, walling-off cankers, and thereby preventing further infection	Family Selection	X	0.11	0.14
Canker alive or tolerance (CANKALIV)	Increased survival even with active cankers	Family Selection	X	0.57	0.26
Bark Reactions (BR)	Increased number of callus formation, walling-off cankers, and thereby preventing further infection	Individual-Tree Selection		0.06	0.24
No spots (NO)[b]	No spot symptoms, no cankers	Individual-Tree Selection		0.15	0.36
Needle shed (NS)	Shedding of infected (spotted) needles in the first fall following inoculation	Individual-Tree Selection		0.07	0.25
Short Shoot (SS)	Isolation of infected needle fascicles; mycelium do not enter branches	Individual-Tree Selection		0.20	0.40

[a] The proportion of the number of individual trees exhibiting the trait divided by the total; values for the family selection traits are based on plot means.
[b] The no-spot trait is the only one to infer immunity—no spotting or canker development are evidenced on a tree; in all other traits, the tree becomes infected but is able to ward off or survive blister rust.

Temperature, photoperiod, water (pH adjusted to 5.5 using phosphoric acid; applied when needed as determined by tray weights), and nutrient availability (Peters Professional® Conifer Grower™ (20N:7P_2O_5:19K_2O), Peters Professional® Conifer Finisher™ (4N:25P_2O_5:35K_2O), magnesium sulfate, calcium nitrate (15.5N:0P_2O_5:0K_2O:19Ca), phosphoric acid, and iron (Fe) were controlled at the time of germination, and during early growth, exponential growth, and the hardening phase. Cleary 3336™ (thiophanate-methyl) fungicide was applied through the irrigation boom to control damping off symptoms caused by *Fusarium* spp.

First and Third Year Greenhouse Data Collection

Survival (presence/absence) and percentage germination were obtained in July 1999 for each seed source. The early season growing regime for the third year of growth was the same as the first 2 years. In preparation for the selection and randomization of seedlings to be inoculated in the fall, all trays of seedlings were moved during the last week of May 2001 from the Quonset-style greenhouse to a fiberglass panel covered greenhouse with a motorized roof vent for venting excess heat during the last week of May 2001. Survival (presence/absence), *Fusarium* spp. infection (presence/absence), terminal damage (presence/absence), and height (mm) were obtained in July 2001 prior to inoculation.

Artificial Inoculation of Treatment Seedlings With Blister Rust

Due to the slower growth of whitebark pine relative to western white pine seedlings, 3-year old rather than 2-year old seedlings were artificially inoculated to have enough surface area of secondary needles for infection (Mahalovich and Dickerson 2004). The target number of seedlings per source was 144 in an effort to pick up some of the resistance traits that are in low frequency, similar to western white pine (Mahalovich 2005). To adequately assess the traits that are thought to be under polygenic inheritance, a minimum of four replications (36 seedlings randomly assigned per replication) are needed to provide reliable estimates. A separate randomization of seedlings, among four replications, was made for the control lots (uninoculated material).

The inoculum source comes primarily from an established *Ribes* spp. garden at Lone Mountain Tree Improvement Area (Idaho). Shrubs included in the garden for whitebark pine inoculations are made up of *Ribes* spp. found in whitebark pine cover types: *R. cereum*, *R. lacustre*, *R. viscosissimum*, and *R. montigenum*. *Ribes* spp. bushes were inoculated in mid to late June with aeciospores collected from active blister rust cankers on whitebark pine across northern Idaho and Montana. Branches from infected plants were used to spread the uredia spores to intensify the infection on the *Ribes* spp. bushes during late July and early August. The garden was irrigated frequently during this period to maintain high relative humidity under the shade cloth structure, which also helps to spread uredia.

Inoculum is collected from the *Ribes* spp. garden when telia horns have ample basidiospore production. The timing of the collection is determined by "plating" sampled leaves in agar petri dishes. Leaves are kept in the petri dishes overnight to allow time for spore drop. The dishes are inspected under a 10X dissection microscope. A decision is made to collect leaves from the garden when the average spore drop count has reached 5 to 10 spores per dish.

Approximately 2,500 *Ribes* spp. leaves were collected for the inoculation screening. The garden was equally divided into 12 sections prior to collection, with the number of leaves per species section determined by the rate of infection and inoculum production present. The goal was to collect at least 200 leaves per section. Leaves were collected no sooner than 24 hours prior to inoculation. Harvested leaves were packaged in groups of 50 in plastic sandwich bags, and a small amount of water was added to the bottom of each bag to keep the leaves moist and to prevent the telia from drying out. Leaves were stored in camp coolers for transportation from the collection point and were refrigerated until used.

An inoculation chamber was created by tightly enclosing a double, hooped framehouse with plastic and canvas to maintain optimum humidity and temperature and to minimize air movement. Soaker hoses placed on the floor were used to maintain humidity in the inoculation structure as close to 100 percent as possible. Humidity was maintained by thoroughly wetting down the interior of the chamber from top to bottom for 24 hours prior to inoculation and by operating soaker hoses in the chamber during the inoculation to keep the wood chips on the chamber floor wet. Temperature was maintained close to 15.5 °C (60 °F) by sprinkling the exterior canvas shell continuously during the inoculation run. Temperature and humidity were monitored by a hygrothermograph placed among the flats of seedlings in the chamber.

Artificial inoculation of the whitebark pine seedlings was scheduled in late summer of the third growing season, when teliospore development on the alternate host was at a maximum. Inoculations began in September 2001, with replications one through four initiated on September 8, 10, 13, and 15, respectively. *Ribes* spp. leaves were randomly placed on screens above the seedlings in the inoculation chamber. Agar-coated microscope slides were placed among the tops of the seedlings to monitor spore drop per cm^2 and percentage germination. When a target spore density of 3,500 to 4,000 spores per cm^2 (22,580 to 25,800 spores per in^2) was reached, leaves were removed from the seedlings. Seedlings were left in the chamber for 48 hours following completion of the inoculation before being returned to the greenhouse. Misting was discontinued at this time to allow seedlings to dehumidify gradually and improve the chances of successful infection of the seedlings by the germinating basidiospores. *Ribes* spp. leaves release basiodospores that germinate and enter needles through the stomates the same day. Needle spots are the first symptom of blister rust infection and are normally visible in a month or two. Later, mycelia move through the plant to the stem and a canker becomes visible in a year to 18 months after inoculation. The seedlings were watered and cultured to maintain health and vigor, but no treatments were applied to enhance growth.

Nursery Bed Data Collection of Treatment and Control Seedlings

All seedlings were hardened off and placed in cold storage at –2 °C (28 °F) in October 2001. During May 2002, seedlings were brought out of cold storage and randomly planted in 36-tree plots in four nursery beds corresponding to the four replications. Transplanted seedlings were watered, fertilized, and weeded as necessary for the duration of the rust-resistance testing. Survival, terminal damage, and needle spot presence were collected on each seedling. In addition, the number of needle spots and fascicle length (mm) were collected on one needle fascicle per tree on all inoculated seedlings in the first inspection (June 2002). The second inspection followed a few months later, where survival, terminal damage, needle spot presence, bark reactions, and canker presence were tabulated (September 2002). The third (September 2003) and fourth (September 2004) inspections involved collecting data on survival, terminal damage, bark reactions and canker presence, and total tree height (cm). Similar data in the same sequence were collected on the control seedlings for completeness.

Freeze-Induced Electrolyte Leakage Test

For this portion of the genetics study, needles were collected in March 2005 from a sample of 55 seed sources using both inoculated and control seedlings. These 55 seed sources included the top 10 resistance sources as defined by a 4-trait index score, the 10 most susceptible sources, and 10 mid-level performers. The remaining 25 sources captured both the geographic and elevational range of the study area. The exact same sources do not comprise both the inoculated and control groups due to differential survival; there are 69 unique sources with 41 in common to both the inoculated and control groups.

Six seedlings from each of the four replications were collected per seed source. Necrotic lesions on needles were extremely rare, and such needles were not used in the samples collected. Visible needle condition was quite healthy for both the inoculated and control seedlings sampled.

Sample preparation of needle tissue for the freeze-induced electrolyte leakage test was patterned after Tinus (2002). The calculation of index of injury for each group data set was based on the averaged control data within a group. The first cold hardiness measurements were completed mid-March 2005. The temperature at which needle tissue exhibited 50 percent index of injury was –28 °C (–18 °F). There were no differences among the three elevations sampled. All of the samples were subsequently tested at –28 °C (–18 °F). These tests were used to provide a point estimate of relative mid-winter cold hardiness for each group based on the relative amount of injury sustained at that one temperature. This estimate for a group will hereafter be referred to as cold hardiness.

Statistical Analysis

Descriptive statistics, ANOVA, and Pearson correlation coefficients were determined using SAS® Software (2003). More detailed information on the materials and methods, techniques, and statistical procedures may be obtained from the senior author.

Results and Discussion

First Year Survival (1999)

At this phase of the study, the individual-tree sources were grouped in trays; there was no blocking by sources. Survival ranged from a minimum of 0.4 percent to a maximum of 93.9 percent, with a mean of 37.7 percent and a standard deviation of 23.9 percent. A one-way ANOVA with seedlots as source of variation yielded significant differences ($P < 0.0001$) among sources (n = 108). Poor germination can, in part, be due to cones being collected before the seeds are fully mature. This commonly occurs in the field when cones have not been sampled and cut to confirm the embryo is occupying at least 90 percent of the central cavity. It can also occur when cones are collected too early to avoid bird and animal predation when wire cages haven't been installed over cone-bearing branches.

Third Year Nursery Evaluation (2001)

Prior to subdividing and randomizing sources among blocks, survival, terminal damage, *Fusarium* spp. presence, and height were scored; all variables were significant ($P < 0.0001$) among sources in the one-way ANOVA. Forty-one of the seed sources (7,147 seedlings) were available for analysis of stocktype using the two types of Super Cells. Significant differences were noted both for terminal damage ($P < 0.003$) and height ($P < 0.0001$) among container types (table 2). The third year average height for the Economy Super Cells was 74 mm (2.9 in), whereas the copper-lined Ray Leach Super Cells was 63 mm (2.5 in). The Economy Super Cell yielded larger seedlings (15 percent increase in height) than the copper-lined Super Cell. At this stage of evaluation, a positive effect with the copper-lining may not be demonstrated because whitebark pine is a slower growing species as compared to other conifers. Also, a better sampling design with equal number of seedlings per stocktype would be more beneficial for making future comparisons.

Blister Rust Resistance Evaluation (2002 to 2004)

Rust resistance traits (table 1) were assessed by observation on each seedling (individual tree selection traits) or were based on the performance of all the seedlings belonging to a seed source (family selection traits). Being able to score inoculated whitebark pine seedlings was not taken lightly. Since we were following the model for western white pine (Mahalovich and Dickerson 2004), we were pleased to have a consistent response to blister rust (spotting, canker, and callus [bark reaction]) development. A preliminary screening of the Shoshone National Forest bulked lot (7425) occurred in a western white pine rust screening (2000 to 2002), so a baseline had been established to proceed at a larger scale.

Overall, the percentage rust resistance among the 108 seed sources after the fourth rust screening was 48 percent (table 3). For the purposes of characterizing blister rust resistance rankings among sources, the traits evaluated were needle lesion frequency, early stem symptoms, bark reaction, and canker tolerance. The relative rust resistance ranking was based on a performance index determined among all sources. Seed source ranks were calculated summing the weighted mean for each trait: bark reaction = 4, needle lesion frequency = 3, early stem symptom appearance = 2, and canker tolerance = 1, respectively (Mahalovich 2005). These rankings were then sorted from best to worst within a seed zone (figure 1) and are reported in table 3, as more resistant sources should be favored for cone collections *within* a zone. No-spot, needle shed, and short shoot traits were included in table 3 for completeness, but are not used to characterize blister rust resistance among seed sources.

All block and seed source main effects were significant ($P < 0.0001$) for all rust traits and height in an ANOVA for the inoculated seedlings (n = 108). Similar results were achieved among the control seedlings (n = 92) for survival and height. Whitebark pine has genetic variation for the rust resistance and height traits evaluated. The differences among seed sources are moderately heritable for rust resistance (0.56) and survival (0.64) and highly heritable (0.85) for 6-year height, which can be improved upon in the future through a selective breeding program. At this time, however,

Table 2—Whitebark pine seedling third-year descriptive statistics and significance probabilities (Pr > F) among stock types (2001).

	Ray Leach Economy Super Cell Cone-tainers™ (n = 7007)		Ray Leach Copper-lined Super Cell Cone-tainers™ (n = 140)		
Trait	Mean	Standard deviation	Mean	Standard deviation	Pr > F between stock types
Survival (%)	95.1	21.6	97.9	14.5	0.133
Terminal Damage (%)	0.7	8.3	2.9	16.7	0.003
Fusarium spp. (%)	1.0	9.8	2.1	14.5	0.166
Height (mm)	73.9	27.8	63.1	24.8	<0.0001

Table 3—Whitebark pine seed sources by zone and relative rankings for rust resistance from (best to worst), cold hardiness, and 6-year height performance (all rankings are based on inoculated seedlings, except where noted for control seedlings *). All sources are individual-tree cone collections, except for 7425, which is a bulk collection made up of at least 20 trees.

Source	Zone	National Forest	State	Lat	Long	Elev (ft)	Rust resistance rank	Cold hardiness rank	6-Yr Height rank
452	BTIP	Nez Perce	ID	45.91	115.713	7140	2	5	80
450	BTIP	Nez Perce	ID	45.91	115.713	7140	10	40	42
644	BTIP	Clearwater	ID	46.302	114.608	7400	11		32
424	BTIP	Salmon	ID	45.468	114.291	7860	21	35	16
734	BTIP	Nez Perce	ID	45.363	116.505	8000	26		72
408	BTIP	Nez Perce	ID	45.634	115.947	8200	35.5		33.5
412	BTIP	Nez Perce	ID	45.634	115.947	8200	37	31	84
336	BTIP	Nez Perce	ID	45.378	116.484	8000	41		101
469	BTIP	Nez Perce	ID	45.706	114.998	8200	42	41	25
643	BTIP	Clearwater	ID	46.302	114.608	7400	49	24	94
739	BTIP	Nez Perce	ID	45.363	116.505	8000	54.5	37*	70
473	BTIP	Nez Perce	ID	45.706	114.998	8200	57.5		86
472	BTIP	Nez Perce	ID	45.706	114.998	8200	64	14	47
505	BTIP	Nez Perce	ID	45.378	116.505	8000	68		92
425	BTIP	Salmon	ID	45.468	114.291	7860	76		103
587	CFLP	Clearwater	ID	46.635	114.859	7200	3	3	81
588	CFLP	Clearwater	ID	46.635	114.859	7200	5	15	51
312	CFLP	Kootenai	MT	47.652	115.74	5650	6	42	57
301	CFLP	Kootenai	MT	47.652	115.74	5650	7	47	27
251	CFLP	Idaho Panhandle	ID	46.999	116.027	5940	13	21	107
589	CFLP	Clearwater	ID	46.635	114.859	7200	18		55
584	CFLP	Clearwater	ID	46.635	114.859	7200	19.5	6*	37
248	CFLP	Idaho Panhandle	ID	47.188	116.048	5880	19.5		54
252	CFLP	Idaho Panhandle	ID	47.014	116.027	5920	25		11
635	CFLP	Clearwater	ID	46.563	114.442	7300	30	10	13
303	CFLP	Kootenai	MT	47.652	115.74	5650	32		48
655	CFLP	Clearwater	ID	46.534	115.004	7000	34		77
630	CFLP	Clearwater	ID	46.563	114.442	7300	43		40
257	CFLP	Idaho Panhandle	ID	46.999	116.027	5800	60.5		79
255	CFLP	Idaho Panhandle	ID	47.014	116.027	5920	63	52	95
637	CFLP	Clearwater	ID	46.563	114.442	7300	78		73
631	CFLP	Clearwater	ID	46.563	114.442	7300	98	38	28
215	CLMT	Deerlodge	MT	46.388	112.191	7600	15		63
69	CLMT	Beaverhead	MT	45.154	113.549	8400	17		49
56	CLMT	Beaverhead	MT	45.154	113.549	8400	29		30
34	CLMT	Beaverhead	MT	45.154	113.549	8400	45.5		19
420	CLMT	Bitterroot	MT	45.72	113.994	8270	56		38
502	CLMT	Bitterroot	MT	46.068	113.801	8040	57.5	17	35
464	CLMT	Bitterroot	MT	46.507	114.224	6470	65		18
26	CLMT	Beaverhead	MT	45.938	113.512	7900	71		2
498	CLMT	Bitterroot	MT	46.068	113.801	8040	72	17*	61
500	CLMT	Bitterroot	MT	46.068	113.801	8040	81	26	75
48	CLMT	Beaverhead	MT	45.154	113.549	8400	83		83
422	CLMT	Bitterroot	MT	45.72	113.994	8270	89	19	24
460	CLMT	Bitterroot	MT	46.507	114.224	6470	99	18	59
535	CLMT	Beaverhead	MT	45.705	112.925	8000	102	4	85
52	CLMT	Beaverhead	MT	45.153	113.549	8400	103	28	97
78	GYGT	Beaverhead	MT	44.818	111.873	8800	47		76
517	GYGT	Targhee	ID	44.554	111.428	8350	52	12	88
7425	GYGT	Shoshone	WY	43.512	109.839	9800	59	16*	58
549	GYGT	Gallatin	MT	45.4	111.279	8600	66	13	60
32	GYGT	Beaverhead	MT	44.818	111.873	8800	73		56
547	GYGT	Gallatin	MT	45.4	111.279	8600	77	2*	52

Continued on next page

Table 3—Continued

Source	Zone	National Forest	State	Lat	Long	Elev (ft)	Rust resistance rank	Cold hardiness rank	6-Yr Height rank
543	GYGT	Gallatin	MT	45.4	111.279	8600	79	22*	45
111	GYGT	Custer	MT	45.042	109.43	8900	80	33	104
95	GYGT	Custer	MT	45.042	109.43	8900	82		50
89	GYGT	Custer	MT	45.042	109.451	9200	84		91
523	GYGT	Gallatin	MT	45.269	111.424	9000	85	11	64
74	GYGT	Custer	MT	45.042	109.451	9200	87		53
512	GYGT	Targhee	ID	44.554	111.428	8350	93	20	78
4	GYGT	Gallatin	MT	45.049	109.95	9600	94		100
546	GYGT	Gallatin	MT	45.4	111.279	8600	100	27	39
530	GYGT	Gallatin	MT	45.269	111.424	9000	104	2	99
41	GYGT	Custer	MT	45.042	109.451	9200	105	9	9
59	GYGT	Custer	MT	45.042	109.555	8900	107	43	68
97	GYGT	Beaverhead	MT	44.818	111.873	8800	108	44	89
663	MSGP	Flathead	MT	48.494	114.341	6000	22		31
270	MSGP	Flathead	MT	48.494	114.341	6000	23		12
676	MSGP	Flathead	MT	48.494	114.341	6000	39		41
669	MSGP	Flathead	MT	48.494	114.341	6000	50		36
271	MSGP	Flathead	MT	48.494	114.341	6000	60.5		69
280	MSGP	Flathead	MT	48.884	114.507	6000	69.5	22	46
267	MSGP	Flathead	MT	48.494	114.341	6500	69.5	29	67
71	MSGP	Lewis & Clark	MT	47.516	112.797	7600	74		14
382	MSGP	Lolo	MT	47.014	114.009	7860	75	39	43
679	MSGP	Flathead	MT	48.884	114.485	6450	88		15
378	MSGP	Lolo	MT	47.014	114.009	7860	90.5		74
85	MSGP	Lewis & Clark	MT	47.835	112.807	7500	92		98
289	SKCS	Colville	WA	48.969	117.109	6800	1	7	102
609	SKCS	Idaho Panhandle	ID	48.379	116.187	6370	4	51	22
340	SKCS	Colville	WA	48.881	117.242	6480	8	46	66
376	SKCS	Lolo	MT	47.014	114.009	7860	9	25	65
481	SKCS	Lolo	MT	47.16	115.249	7050	12		1
690	SKCS	Idaho Panhandle	ID	46.171	116.735	5430	14		23
496	SKCS	Lolo	MT	47.086	114.576	7420	16		105
612	SKCS	Idaho Panhandle	ID	48.379	116.187	6370	24		71
594	SKCS	Lolo	MT	47.522	115.699	6150	27	49	29
337	SKCS	Idaho Panhandle	ID	48.826	116.599	6800	28		17
627	SKCS	Idaho Panhandle	ID	48.84	116.512	6700	32	54	20
484	SKCS	Lolo	MT	47.16	115.249	7050	32		9
329	SKCS	Kootenai	MT	47.826	115.385	5650	35.5		8
296	SKCS	Idaho Panhandle	ID	48.855	116.469	5820	38		82
603	SKCS	Idaho Panhandle	ID	48.379	116.187	6370	40	53*	2
595	SKCS	Lolo	MT	47.522	115.699	6150	44		5
440	SKCS	Lolo	MT	47.158	114.366	6960	45.5		87
490	SKCS	Lolo	MT	47.086	114.576	7420	48	6	90
334	SKCS	Kootenai	MT	48.97	115.842	7200	51	48	21
477	SKCS	Lolo	MT	47.16	115.249	7050	53	8	6
297	SKCS	Idaho Panhandle	ID	48.855	116.469	5720	54.5	55	93
623	SKCS	Lolo	MT	47.753	114.85	6140	62	36	10
325	SKCS	Kootenai	MT	47.953	115.556	6000	67	30	7
626	SKCS	Idaho Panhandle	ID	48.84	116.512	6700	86	42*	33.5
314	SKCS	Kootenai	MT	47.826	115.385	5700	90.5	53	3
351	SKCS	Colville	WA	48.707	118.471	7135	95	50	106
480	SKCS	Lolo	MT	47.16	115.249	7050	96	41*	4
434	SKCS	Lolo	MT	47.158	114.366	6960	97	32	44
349	SKCS	Colville	WA	48.707	118.471	7137	101		108
617	SKCS	Lolo	MT	47.753	114.85	6140	106	45	26

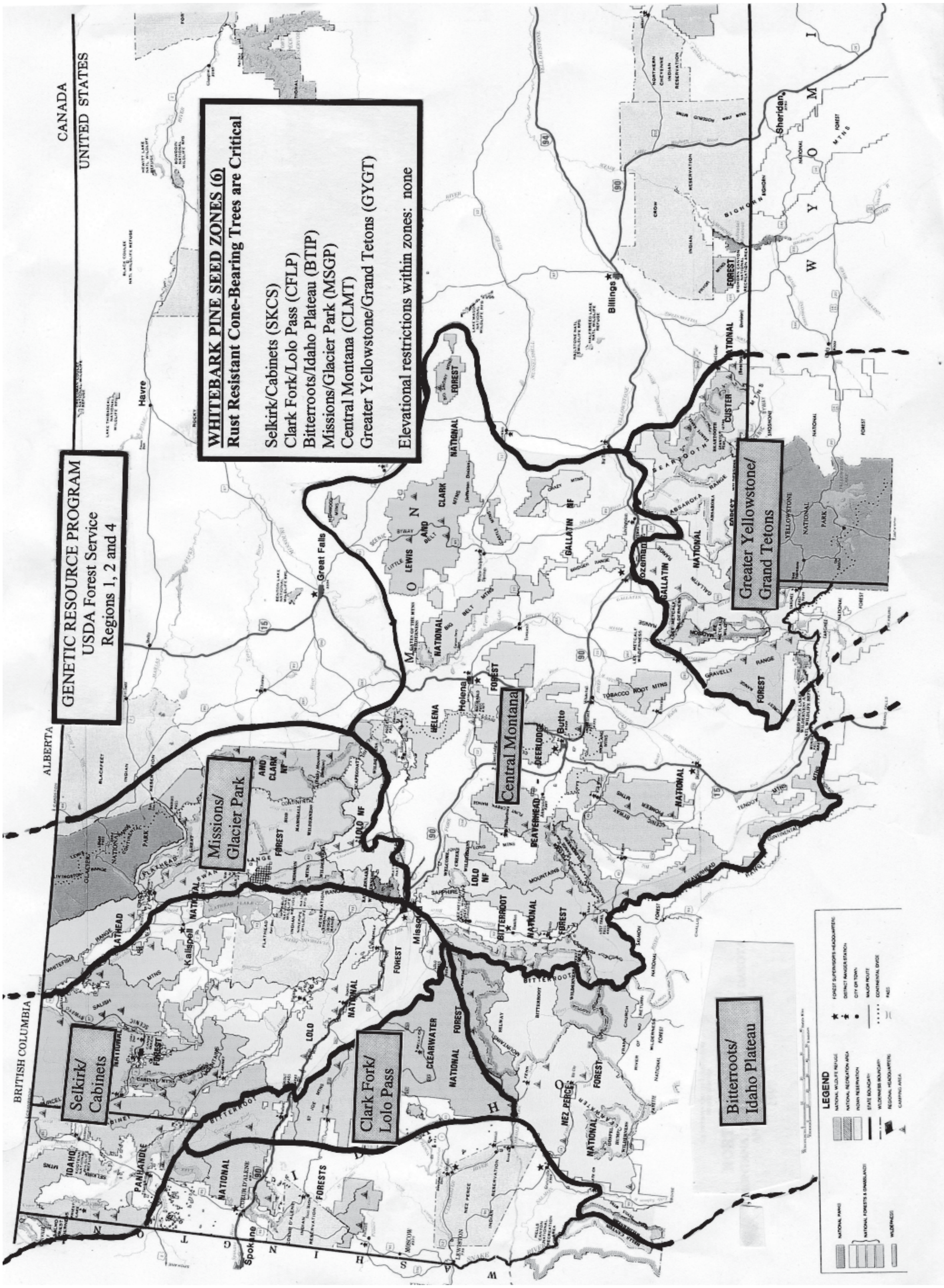

Figure 1—Whitebark pine seed zones for the Northern Rockies.

there are no plans for a breeding program in whitebark pine (Mahalovich and Dickerson 2004); current plans are to work on the selection and testing (rust screenings) and establishing small-scale seed orchards (about 1.5 ac [0.6 ha] in size). The height rankings found in table 3 were derived from the inoculated seedlings. Overall, blister rust resistance increases from southeast to northwest (figure 2).

Cold Hardiness (2005)

Prior to measuring the index of injury for each seed source, a control line (benchmark) at 50 percent injury was established. This benchmark was consistent among both inoculated and control seedlings and across a sample of low, moderate, and high elevation sources, so there was no difference in the amount of leakage other than from freezing. Only seed source as a main effect (n = 55) was significant ($P < 0.0001$) for index of injury in an ANOVA for the inoculated seedlings; blocks were not significant. The differences among seed sources were moderately heritable for cold hardiness (0.50). Both block and seed sources as main effects were significant ($P < 0.0001$) for index of injury in an ANOVA for the control seedlings. There was a slight difference among seedling types; in other words, blister rust appears to have impacted needle tissue hardiness. The cold hardiness ranking for the inoculated seedlings ranged from 50.8 to 81.3. The cold hardiness range for the control seedlings was 38.3 to 76.6. Overall, the control seedlings were more cold hardy than inoculated seedlings (average score of 58 versus 63). We anticipate providing more absolute values and a more detailed assessment. Focusing on seed sources for cone collections, relative rankings of cold hardiness among the 55 samples are found in table 3 (lower scores are more cold-hardy). These measurements used to determine cold hardiness rankings are point estimates sampled in late winter. Additional work is recommended to determine if late winter/early spring cold hardiness is more critical for whitebark pine, as in western larch (*Larix occidentalis*) (Rehfeldt 1995) or if late summer/early fall cold hardiness is a more important adaptive measure, as in Douglas-fir (*Pseudotsuga menziesii*) (Rehfeldt 1979).

Trait Correlations

Early in the whitebark seedling's life, there does not appear to be a physiological trade-off between allocating resources for rust resistance at the expense of growth; however, trees with more rust resistance are slightly less cold hardy, although not statistically significant. Height has an unfavorable and weak correlation with cold hardiness (taller seedlings have a larger index of injury). Taller trees are more rust resistant and are *slightly* less cold hardy.

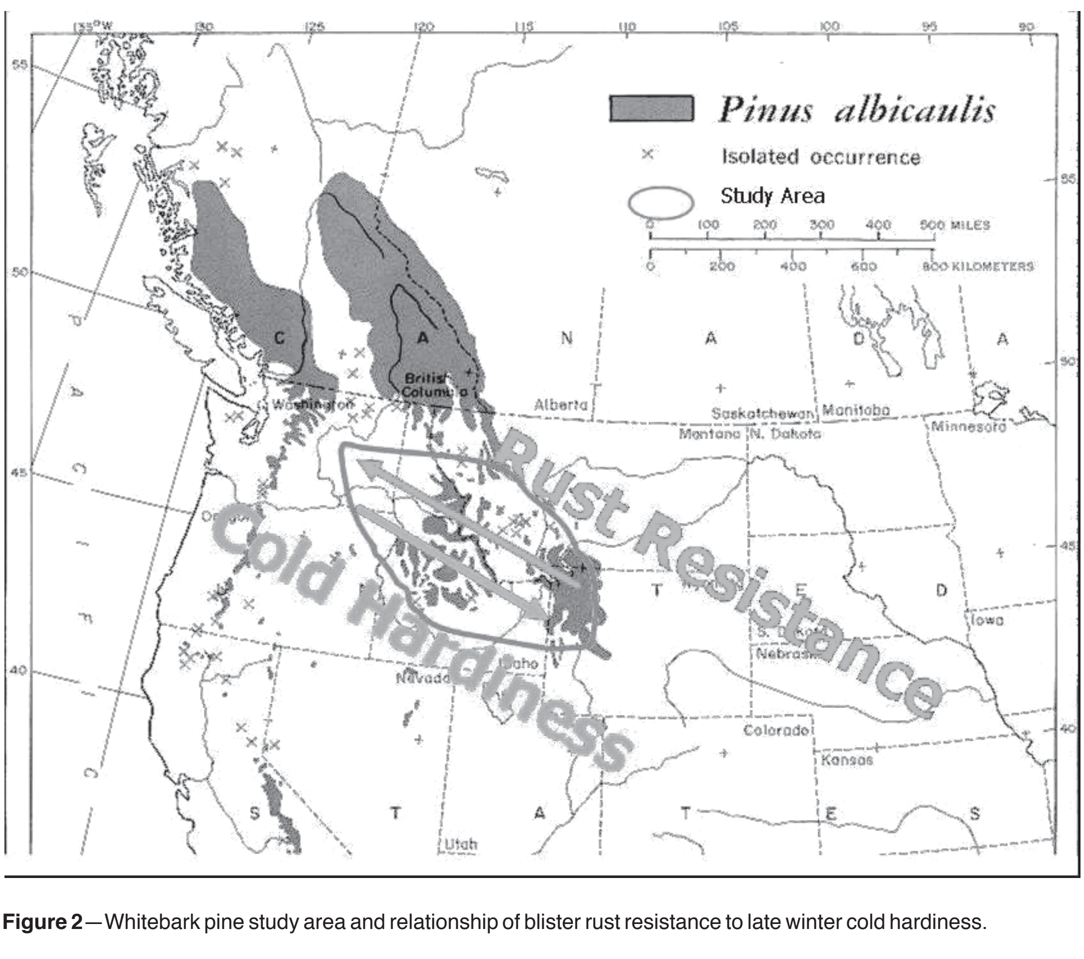

Figure 2—Whitebark pine study area and relationship of blister rust resistance to late winter cold hardiness.

These correlations can be managed by choosing seed sources within a seed zone that possess both desirable rust resistance and cold hardiness levels. Unfavorable correlations can also be handled in designing breeding zones and choosing selection methods in tree improvement programs that mediate these opposing trends. Even though cold hardiness decreases from southeast to northwest (figure 2), not all seed sources have poor rust resistance; for example, source 587 (Clearwater National Forest, seed zone CFLP), source 452 (Nez Perce National Forest, seed zone BTIP), and the number one rust resistant source 289 (Colville National Forest, seed zone SKCS) are relatively cold-hardy even though they are in the northwest portion of the region (table 3).

WBP Planting Strategies For Restoration

It is possible to proceed with immediate restoration and wildlife habitat improvement through planting since we have identified both rust resistant and cold hardy seed sources within six of the seed zones studied. A summary of the key findings is presented in the following planting recommendations:

1) Choose rust resistant sources within a seed zone (table 3).

2) Ensure that cone collections have a minimum of 20 cone-bearing trees separated by 200 ft (61 m) in distance to minimize any negative effects of inbreeding.

3) There are no elevation restrictions on seed transfer within a seed zone.

4) When blister rust infection levels vary within a zone, seeds collected for immediate rehabilitation efforts should not be moved from areas with low (<49 percent) to moderate (50 to 70 percent) infection levels to outplanting sites with higher infection levels (>70 percent) (Mahalovich and Dickerson 2004). Seeds collected from phenotypically resistant trees in areas with high infection levels are suitable for outplanting on sites with low, moderate, or high infection levels (Mahalovich and Hoff 2000).

5) The top three resistant seed sources per seed zone should be considered an effective cone collection strategy for a 10-year planning window. The next 10-year planning period should focus on a minimum of three *new* collection areas in order to broaden the genetic base used in outplanting programs over time. This assumes that the USDA Forest Service Northern and Intermountain Regions Whitebark Pine Genetic Restoration Project achieves stable funding to proceed with rust screening of the additional 650 plus trees described in Mahalovich and Dickerson (2004).

6) When selecting stocktypes, there appears to be no advantage to using copper-lined containers.

7) When planting in swales or frost pockets, choose cold-hardy sources that are rust resistant (table 3).

8) Field monitoring of outplanted whitebark pine seedlings shows a favorable advantage to providing a microsite regardless of slope, aspect, swales, or frost pocket conditions. We recommend planting seedlings next to stumps, logs (figure 3), and, if none are available, use rocks (figure 4) or shade cloths. Note in figure 3 the relative heights of the shorter seedling outplanted in the open, the mid-sized seedling near a log, and the tallest seedling adjacent to the downed log. The microsite is reminiscent of Clark's nutcracker who cache seeds near the base of trees, roots, logs, rocks, plants, or in cracks and fissures in trees and logs (McCaughey and Tomback 2001). The microsite is thought to provide shade and increased soil moisture retention during early establishment.

Figure 3—Microsite example using logs next to planted whitebark pine seedlings on the Clearwater National Forest (Bob Grubb, Forest Tree Improvement Coordinator and Lenore Seed Orchard Manager in photo).

Figure 4—Microsite example using a rock next to a planted whitebark pine seedling on the Caribou-Targhee National Forest.

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