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

Mary F. Mahalovich
Karen E. Burr
David L Foushee

Mary F. Mahalovich is Regional Geneticist, USDA Forest Service, Northern, Rocky Mountain, Southwestern, and Intermountain Regions, 1221 S Main Street, Moscow, ID 83843; telephone: 208.883.2350; e-mail: mmahalovich@fs.fed.us. Karen E. Burr is Horticulturist, USDA Forest Service, Coeur d'Alene Nursery, 3600 Nursery Road, Couer d'Alene, ID 83814; telephone: 208.765.7429; e-mail: kburr@fsfed.us. David L. Foushee is Tree Improvement Horticulturist, USDA Forest Service, Coeur d'Alene *Nursery,* 3600 Nursery Road, Couer d'Alene, ID 83814; telephone: 208.765.7394; e-mail: dfoushee@fs.fed.us

In: Riley, L . E.; Dumroese, R. K.; Landis, T. D., tech. coords. 2006. National Proceedings: Forest and Conservation Nursery Associations-2005. Proc. RM RS-P-43. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, 160 p. Available at: http://www.rngr.net/nurseries/publications/proceedings

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 In = 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^{TM} (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

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 lox 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 Lelia 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 –2ºC (28 ºF) 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 midlevel 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 in dex 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 –28ºC (-18 ºF). There were no differences among the three elevations sampled. All of the samples were subsequently

tested at –28 ºC (-18 ºF). These tests were used to provide a point estimate of relative midwinter 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 notbe 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 I) 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-tainersTM (n 7007)		Ray Leach Copper-lined Super Cell Cone-tainers™ (n = 140)		P r > F between stock types
Trait	Mean	Standard' deviation	Mean	Standard deviation	
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	3T	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	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	ID	47.188	116.048	5880	19.5		54
252	CFLP	Idaho	ID	47.014	116.027	5920	25		11
635	CFLP	Clearwater	ID	46.563	114442	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	ID	46.999	116.027	5800	60.5		79
255	CFLP	Idaho	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	CLM	Deerlodge	MT	46.388	112.191	7600	15		63
69	CLM	Beaverhead	MT	45.154	113.549	8400	17		49
56	CLM	Beaverhead	MT	45.154	113.549	8400	29		30
34	CLM	Beaverhead	MT	45.154	113.549	8400	45.5		19
420	CLM	Bitterroot	MT	45.72	113.994	8270	56		38
502	CLM	Bitterroot	MT	46.068	113.801	8040	57.5	17	35
464	CLM	Bitterroot	MT	46.507	114.224	6470	65		18
26	CLM	Beaverhead	MT	45.938	113.512	7900	71		2
498	CLM	Bitterroot	MT	46.068	113.801	8040	72	17*	61
500	CLM	Bitterroot	MT	46.068	113.801	8040	81	26	75
48	CLM	Beaverhead	MT	45.154	113.549	8400	83		83
422	CLM	Bitterroot	MT	45.72	113.994	8270	89	19	24
460	CLM	Bitterroot	MT	46.507	114.224	6470	99	18	59
535	CLM	Beaverhead	MT	45.705	112.925	8000	102	4	85
52	CLM	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
742	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