Whitebark Pine Genetic Restoration Program for the Intermountain West (United States)

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Abstract—A strategy to restore whitebark pine communities is presented that emphasizes genetic resistance to white pine blister rust (Cronartium ribicola Fisch.) and mountain pine beetle (Dendroctonus ponderosae Hopkins), in combination with an active tree planting program. Early and active intervention may prevent listing of whitebark pine under the Endangered Species Act and further aid in the successful recovery of the grizzly bear (Ursus arctos horribilis). The restoration program initiated in 2001 includes a multi-State effort (Idaho, Montana, Oregon, Nevada, Wyoming, and Washington) designating permanent leave trees, emphasizing clean trees in high blister rust areas or areas with a high incidence of mountain pine beetle, or areas where both conditions are present. Cone collections from these trees will provide an immediate seed source for fire restoration, reforestation, ex situ genetic conservation, and seedlings to be screened for blister rust resistance. Pollen will be collected for genetic conservation and to advance blister rust resistance in seed and breeding orchards. Data generated from the rust screenings will identify whitebark pine seed sources that provide high levels of blister rust resistance and provide information needed to refine seed transfer guidelines. Leave trees elevated to elite-tree status, as identified by their rust-resistant progeny in the rust screenings, will serve as a seed source for operational collections and seed trees for natural regeneration. Survivors from the blister rust screening will be planted in clone banks for genetic conservation purposes, to serve as donors for future seed orchard establishment, and to facilitate selective breeding for blister rust resistance.

Key words: white pine blister rust resistance, fire restoration, genetic conservation, seed transfer guidelines.

Introduction

Whitebark pine, a keystone species in upper and subalpine ecosystems, provides a food source for grizzly bear, Clark’s nutcracker (Nucifraga columbiana), and red squirrels (Tamiasciurus hudsonicus). It is also a foundation species for protecting watersheds as it tolerates harsh, windswept sites that other conifers cannot, the shade of its canopy regulates snowmelt runoff and soil erosion, and its roots stabilize rocky and poorly developed soils (Tombback and Kendall 2001).

The native pathogen, limber pine dwarf mistletoe (Arceuthobium cyanocarpum (A. Nelson ex Rydberg) Coulter & Nelson) and the exotic pathogen, white pine blister rust, are contributing to the overall decline of the species. The parasitism of dwarf mistletoe impacts cone and seed production, reducing the reproduction potential of whitebark pine in severely infested stands (Taylor and Mathiason 1999). White pine blister rust rapidly kills small trees, impeding successful regeneration. Blister rust infections in larger trees can persist a long time and are frequently found in the upper crown, reducing a tree’s cone-bearing potential. Whitebark pine trees that survive blister rust infections are further threatened by mountain pine beetle attacks.

Wildfire occurrence aids in the preparation of a seed bed for natural regeneration. Fire suppression has reduced the role of fire in regeneration of pure whitebark pine stands and has allowed successional replacement of subalpine fir (Abies lasiocarpa (Hook.) Nutt.), lodgepole pine (Pinus contorta Dougl. ex Loud.) and Engelmann spruce (Picea engelmannii Parry ex Engelm.) in mixed-conifer stands. Careful control is needed to reintroduce fire into high elevation ecosystems. Uncontrolled wildfire can destroy young whitebark pine regeneration and kill trees of cone-bearing age, which will limit the food supply for dependent wildlife and cause loss of future seed sources for restoration purposes.

High elevation ecosystems are at high risk because one or two species of white pines are usually dominant (McDonald and Hoff 2001). Because the loss of mature whitebark pine is occurring so rapidly, often in the absence of successful regeneration, there has been a pronounced loss of whitebark pine cover type. When only thinning and prescribed fire are utilized to promote vigorous stands of western white pine (Pinus monticola Doug. ex D. Don), this has led to increased blister rust infection levels by opening up stands and encouraging Ribes spp. establishment (Schwandt and others 1994). Successful natural regeneration is dependent upon sufficient blister rust resistant seed available on site. This is due to the unique seed dispersal and seed caching by Clark’s nutcrackers (Tomback and Schuster 1994) and red squirrels.
The 2000 fire season burned 929,000 ha on USDA National Forest System lands. Much of the fire devastation occurred in high elevation ecosystems, resulting in the destruction of both diseased and healthy whitebark pine trees.

Emergency National Fire Plan funding was made available in 2001 to initiate a landscape-level approach to restoring whitebark pine over the next 5 years on National Forest System lands in Idaho, Montana, Nevada, and Wyoming. Adjacent National Forests in Washington and Oregon were invited to participate. Glacier, Grand Teton, and Yellowstone National Parks, facing similar management challenges and stringent restoration policies (Kendall 1994), were also invited to participate. The scope of the program is based on cooperators whose landholdings are high elevation sites typically found in Federal ownership. The multi-State, multiagency collaboration forged in this endeavor provides a unified front to increase the likelihood of favorable outcomes in our restoration efforts, and a synergy that has been difficult to achieve by any one administrative unit or special project in the past.

**Project Goals**

The short-term goals over the next 5-year period are: (1) operational cone collections for planting burned areas, and (2) plus-tree identification and individual-tree cone collections for rust screenings and genetic conservation. These activities will facilitate identification of whitebark pine populations at most risk due to blister rust (more than 70 percent infection), which may require additional intervention to stabilize their survival. Field personnel will also become more familiar with the distribution of whitebark pine, which will provide land managers current information on the species distribution (Little 1971) and associated blister rust infection levels and mountain pine beetle infestations across the landscape. These data will also be used to adjust the number of plus-trees needed per zone and to develop a database for a seed transfer expert system.

Over the long-term, seedlings from the plus-tree selections will reveal patterns of genetic variation in survival, blister rust resistance, and early growth in rust screening trials. Data obtained from the rust screenings will help identify the presence or absence of various blister rust resistance mechanisms (Mahalovich and Eramian 1995) and their relative frequency among populations. The performance of the rust-resistant progeny will also be used to rank the original plus-trees. Those with high rankings (elite trees) will be identified as scion and pollen donors for seed orchard and clone bank establishment.

**Implementation Plan**

### Cone Collections for Fire Rehabilitation

National Forests and Parks with immediate restoration needs should use the current seed zone boundaries to estimate their seed needs (fig. 1). There are no elevational restrictions on seed transfer within a seed zone. When blister rust infection levels vary within a zone, seeds collected for immediate rehabilitation efforts should not be moved from areas with low (less than 49 percent) to moderate (50 to 70 percent) infection levels to planting sites with higher infection levels (more than 70 percent). Seeds collected from phenotypically resistant trees in areas with high infection levels are suitable for planting on sites with low, moderate or high infection levels (Mahalovich and Hoff 2000).

Operational cone collections should be from no fewer than 20 individuals separated by 67 m within a zone to ensure a broad genetic base in the seed lot. This bulked seed lot collected from similar rust infection sites is referred to as a tree-seed zone or bulked collection.

Additional improvement in insect and disease resistance and growth can be achieved by collecting from above-average stands with more than 50 reproductively mature trees per 0.5 ha, emphasizing collections from a minimum of the 20 best trees. This bulked seed lot is referred to as a seed collection stand.

Moreover, communities with high blister rust infection or mountain pine beetle infestations, with at least 50 clean, reproductively mature trees per 0.5 ha, could be cultivated as a seed production area. This concept offers even more improvement, by first selecting an above-average stand, followed by removal of undesirable trees with insect and disease problems and poor growth and form, improving the genetic base of both the seed and pollen parents. These potential seed production areas will provide the most promising seed source for immediate cone collections until a grafted seed orchard of proven rust-resistant donors can be established and cultured for cone production.

**Identifying Phenotypically Superior Individuals**

An effective restoration strategy in whitebark pine includes components related to patterns of genetic variation, particularly to blister rust. Restoration efforts may be hampered if the assumption is made that whitebark pine and western white pine have a similar genetic response to blister rust. One key difference is that percent infection is higher in whitebark than western white pine (Bingham 1972, Hoff and others 1994, McDonald and Hoff 2001). Until more information becomes available on the biology and genetics of whitebark pine and blister rust in the Inland West, the best model to develop blister rust improvement in whitebark pine is the western white pine protocol (Mahalovich and Eramian 1995). Several modifications have recently emerged regarding the western white pine protocol and in the recommended breeding plan to develop resistance in whitebark pine put forth by Hoff and others (1994). The revised protocol follows.

Plus-tree selections (that is, designation of permanent leave-trees) are based on existing seed zones (fig. 1). Assignments within zones facilitates broad sampling among National Forests and Parks, emphasizing broadly adaptable populations for blister rust resistance development and isolated populations supporting unique gene frequencies or adapted gene complexes for gene conservation. If the target seed orchard size is 30 unrelated individuals, sufficient candidate trees must be identified within a zone to assure finding several genes for blister rust resistance in the rust screenings.
Approximately 100 plus-trees are assigned in each seed zone relative to the number of hectares of whitebark pine occurring on National Forests and Parks (Little 1971). The state of Nevada is comprised primarily of isolated populations with an expectation of 50 plus-trees for that zone. The outlier populations in northeastern Oregon are typically considered as part of the western range of whitebark pine (McCaughey and Schmidt 2001); however, these populations are also in proximity to the Bitterroots/Idaho Plateau seed zone boundary (fig. 1). Until more information becomes available on these populations, progeny from northeastern Oregon should be evaluated in rust screenings alongside progeny from both the Bitterroots/Idaho Plateau and the Nevada seed zones.

The total base population across all zones is 650 trees. The base population may seem small as compared to the 3,100 plus-trees in the western white pine tree improvement program (Mahalovich and Eramian 1995). The effective population size in western white pine is actually less than 3,100 plus-trees, as field validation has shown some trees separated by as little as 10 m will increase the probability that they are related. The goal is to have a moderate number of trees per zone to assure finding several genes for resistance. Problems in too small a population size within a zone may arise if 30 rust-resistant elite trees are not identified in a rust screening.
The western white pine program required 900 sound seeds per plus-tree; 300 to be set aside for gene conservation and the remaining to be sown to provide 144, 2-year-old container seedlings for rust screenings (Mahalovich and Eramian 1995). For whitebark pine, field units have been asked to collect 1,800 wind-pollinated seed per tree, 300 for gene conservation, and the remaining for rust screenings. Wire cages are recommended to protect the cones from bird and squirrel predation to achieve the target number of seeds per tree. The wire cages should be installed during June, on branches bearing second-year conelets. The increased number of seeds per tree are needed to compensate for low germination rates from sowing seedlots that have been in extended cold storage from the early 1990s (Burr and others 2001). Efforts are under way with the USDA Forest Service National Tree Seed Laboratory to study the special germination and seed storage requirements of whitebark pine, to make a seed bank a more promising gene conservation tool in the future.

Whitebark Pine Plus-Tree Selection Criteria

Stand-Level Selection Criteria—The stand selection criteria were relaxed for whitebark pine, emphasizing blister rust infection levels instead of mortality levels (table 1). If average mortality levels were followed, as was recommended for western white pine, almost no whitebark pine stands would qualify for plus-tree selections. Mortality levels can reach upwards of 90 percent or higher in whitebark pine stands in the Selkirk-Cabinet seed zone. Where field units do not support stands of whitebark pine (for example, more than 50 trees per 0.5 ha) and have dispersed trees in mixed-conifer settings, field personnel should move forward to the individual-tree selection criteria.

The average infection level for the target stand is determined by carefully counting both live and dead cankers from a representative sample of 100 living or dead trees. Presence or absence of cankers (bole and branch) from the 100-tree survey is used to determine the overall stand infection level. Actual counts should be made for main-bole cankers, whereas branch cankers can be estimated and grouped in the following categories: 0=no cankers present, 1 to 9 cankers, 10 to 20, 21 to 40, 41 to 75, 76 to 150, 150+ cankers. The combined total of main bole cankers and estimated branch cankers is equal to the number of cankers per tree. The average number of cankers per tree for the 100-tree survey then yields the stand average. When rust infection is heavy (some 90 percent), allowances are made for the possible presence of difficult-to-see or undetectable cankers (for example, flagging, dead tops, dead branches, and animal damage with extensive sap on the main bole are assumed to be due to a canker).

Each area should be more than 25 years of age and the average tree height around 10 to 35 m. This will increase the likelihood that the stand will have had at least 25 years of exposure to blister rust, be of cone bearing age, be producing pollen, and be climbable. A moderately open stand density is desirable so the target plus-trees are easy to examine from the ground, have persistent branches at ground level to facilitate climbing, and have full crowns for better cone-bearing potential (Hoff and McDonald 1980).

When rust infection levels are low (less than 50 percent) and whitebark pine grows in either a mixed- or pure-stand setting, field units should proportionally balance the number of living or dead whitebark pine, well represented throughout the stand.

Table 1—Whitebark pine plus-tree selection criteria.

<table>
<thead>
<tr>
<th>Stand level criteria</th>
<th>Individual-tree level</th>
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<tbody>
<tr>
<td>Vigorous and representative of the species</td>
<td>Dominant or co-dominant trees</td>
</tr>
<tr>
<td>Habitat type where species normally occurs</td>
<td>Minimum of 100 m between selected trees to avoid relatedness</td>
</tr>
<tr>
<td>Provide a broad sample of both the geography and range of elevations</td>
<td>Free of insects and diseases</td>
</tr>
<tr>
<td>Overall composition has a high proportion of living or dead whitebark pine, well represented throughout the stand</td>
<td>Have a history or the potential to bear cones</td>
</tr>
<tr>
<td>Uniformly and heavily infected with blister rust (10 or more cankers per tree on the average)</td>
<td>Be within 100 to 200 m from the nearest road or trail</td>
</tr>
<tr>
<td>Confirmed blister rust infection of 90 percent or higher in uniform stands</td>
<td>No more than three of the best candidates in any given stand</td>
</tr>
<tr>
<td>Stands with 50 to 90 percent rust infection, limit selected trees to no more than five cankers</td>
<td>No squirrel cache cone collections</td>
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1 Spacing between plus-trees (100 m) differs from spacing requirements in operational cone collections (67 m).
of selections between the two stand types. Likewise, if field units have both concentrated stands and sparsely distributed whitebark pine, plus-tree collections should be proportionally balanced based on the number of hectares occurring in both types of tree densities.

**Individual-Tree Selection Criteria**—Each plus-tree should be relatively free of blister rust when compared to the overall infection level in the stand. Allowable infection levels for each plus-tree (table 2) are modeled after Hoff and McDonald (1977, 1980). The presence or absence of cankers is determined by examining each tree both from the ground with binoculars and by climbing the tree and examining each individual whorl. Though desirable, based on the preliminary field reports, accurate canker counts are difficult in whitebark pine because of the high levels of infection, sap weeping from cankers and animal damage (chewing), as compared to western white pine.

Three growth forms are acceptable in whitebark pine: single-stem, erect; multiple-stem, erect; and krummholz. Dominant or co-dominant trees are preferable, but the multiple-stem, erect or krummholz categories may lend themselves more to the intermediate or suppressed crown classes. In contrast to western white pine, the acceptable growth form is the single-stem, erect form, or the timber archetype in the dominant or co-dominant crown class.

Each tree should be free of insects, particularly mountain pine beetle, and other diseases such as limber pine dwarf mistletoe, as these characteristics are likely inherited and passed onto their progeny. Squirrel-cache cone collections should be avoided because of unknown parentage and because seeds have come in contact with forest litter and soils, increasing the likelihood of seed-borne fungi *Fusarium* spp., *Sirococcus strobilinus* and the snow bank or cold fungus, *Calocypha fulgens* (Kolotelo and others 2001, Hoff and Hagle 1990).

Each tree should be within 100 to 200 m from the nearest road or trail, unless intervening vegetation is sparse enough so that longer lines of sight are possible, to facilitate caging of branches to protect cone crops from bird predation and for ease of relocation. When plus-trees are easily accessible by road or trail, the possibility exists to use cherry pickers or man-lifts to collect cones from the upper portion of the crown.

Care should be taken to avoid collections from limber pine, when whitebark and limber pine are intermixed on the same national forest or park. The operational cone collection guidelines for whitebark pine (Mahalovich and Hoff 2000) provide additional information on how to distinguish the two species by cone morphology, strobilus color, and pollen catkin color.

### Table 2—Acceptable canker limits for individual plus-trees based on stand averages.

<table>
<thead>
<tr>
<th>Stand average (cankers/tree)</th>
<th>Plus-tree limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 to 20</td>
<td>No cankers</td>
</tr>
<tr>
<td>21 to 40</td>
<td>1 canker</td>
</tr>
<tr>
<td>41 to 75</td>
<td>2 cankers</td>
</tr>
<tr>
<td>76 to 150</td>
<td>3 cankers</td>
</tr>
<tr>
<td>151+</td>
<td>4 or 5 cankers</td>
</tr>
</tbody>
</table>

**Blister Rust Screening Trials**

A rust screening will let us know how successful our restoration efforts may be by identifying the amount of genetic variation present in survival and disease resistance and by quantifying how much of that variation occurs among or within stands.

The progeny of 200 plus-trees can be reliably handled in a rust screening, allowing approximately two seed zones to be tested at a time. A bulked check lot of untested seed from existing whitebark pine seed lots will need to be constructed upfront, to facilitate comparisons among the plus-trees. Rust screening scheduling will depend on how quickly each field unit completes its plus-tree selections within a zone. The goal is to sow a rust screening trial by 2005.

Modifications in the composition of aeciospore samples are recommended as a conservative course of action for inoculating *Ribes* spp. in the rust screening trials. Low levels of genetic differentiation exist among samples of *C. ribicola* collected from eastern white pine (*Pinus strobus* L.) in eastern North America (Et-touil and others 1999) and among *C. ribicola* samples collected from western white pine in western North America (Kinloch and others 1998). Little is known however, about specific races of blister rust in the Inland West in western white or whitebark pine. One exception is the identification of yellow and red-spotting races occurring on western white pine (McDonald 1978), with one type not necessarily more virulent than the other. *Ribes* spp. leaves used in the inoculations should be treated with aeciospores collected from cankers on whitebark pine, in the event there are different rust populations in white- bark and western white pine communities. Aeciospores will be collected 1 to 2 years prior to rust screening from a representative sample across all seed zones. State-to-state plant inspection regulations may prohibit the transfer of spore collections across state lines, so further modifications in the rust screening protocol may be warranted in the future.

This conservative approach is also appropriate when considering the alternate host, because a different mix of *Ribes* spp. occurs in whitebark pine communities (for example, *Ribes lacustris, R. viscossissimum*, and *R. montigenum*) than in western white pine (for example, *R. cereum, R. nigrum* and *R. hudsonianum* var. *petiolare*). A Ribes garden for whitebark pine inoculations was established at Lone Mountain Tree Improvement Area, Idaho Panhandle National Forests in 2000.

Hoff and others (1994) recommended inoculating 2-year old whitebark pine seedlings. Due to the slower growth rates of whitebark pine as compared to western white pine, these rust screenings will use 3-year old container seedlings in order to have enough top shoot and secondary needles to be challenged with inoculum.

During the inoculation procedure, basidiospores will be delivered at a target rate of 3,500 spores per cm². Previous rust screenings of whitebark pine using a rate recommended for western white pine have shown a delivery of 6,000 spores per cm² to be too high, killing most of the seedlings in a given block (Mahalovich unpublished data).

Four rust inspections will be performed in each trial. The first and second inspections will occur 9 months and 12 months, respectively, after inoculation. The third and fourth inspections will occur during September in subsequent
years. Overall, the four rust inspections span a 3-year period. Data collected during each inspection will be the same as data acquisition for western white pine trials (Mahalovich and Eramian 1995).

Last, to minimize cross-contamination of susceptible seedlings and inoculated Ribes spp. leaves, and the possible introduction of virulent rust races between species, a recommended quarantine procedure is to avoid inoculating western white and whitebark pine seedlings in the same calendar year at the same location (Coeur d’Alene Nursery, Coeur d’Alene, Idaho).

Data Applications

Refine Seed Transfer Guidelines—Seed transfer (Mahalovich and Hoff 2000) is currently based on seed zones (fig. 1) driven by major mountain ranges and existing knowledge of blister rust infection levels in populations of whitebark pine (Hoff and others 1994). A better approach to seed transfer is to develop guidelines based on phenological and blister rust resistance data. Early genetic studies using isozymes point to low levels of genetic variation among and within stands of whitebark pine (Lanner 1982, Jorgensen and Hamrick 1997, Brueederle and others 1998). Richardson (2001) examined uniparentally inherited mitochondrial (mt)DNA and chloroplast cp(DNA) microsatellites (cpSSRs) to examine population genetic structure from 38 coastal and interior populations of whitebark pine. Analysis of Molecular Variance (AMOVA) groups based on an exact test suggested four zones among Inland West populations: Sierra Nevada Mountains, Yellowstone, central Idaho, and northern Idaho. Data obtained from the sites sampled for plus-trees (blister rust infection levels) and the rust screening trials will validate whether the existing seed zones could be combined into four zones, determine where the geographic boundaries should be drawn, and provide a model for predicting safe seed transfer for individual seed lots using a seed transfer expert system. Zone boundaries will be revised before proceeding with the establishment of seed orchards and clone banks.

Seed Orchard Establishment and Design—Each plus-tree will be ranked based on the performance of its progeny in the rust screening trials using the same evaluation criteria established in western white pine (Mahalovich and Eramian 1995). Preliminary rust screenings have shown whitebark pine seedlings to exhibit rust resistance responses much like the other five-needle pines but at different frequencies (Hoff and others 1980, Hoff and Hagle 1990). The higher-ranking parent trees will be revisited to collect scion to establish production seed orchards within each zone. Sowing and growing of rootstock will be coordinated with the completion of each rust screening. Until these orchards reach reproductive maturity, the rankings of the plus-trees can be matched to their native stands to identify promising cone collecting areas (seed collection stand or seed production area) not previously identified during 2001 through 2005, to meet more immediate seed needs for resistant planting stock.

Data collected from the rust screenings will also be used to facilitate seed orchard design and seed deployment strategies by resistance mechanism(s). This strategy of using patterns of genetic variation and deploying more than one resistance mechanism on any given hectare makes it unlikely a new, more virulent race of rust will develop in planted stock (Mahalovich and Eramian 1995).

Pollen can be a limiting factor in immature pine orchards, when the goal is to obtain enough sound seed from a broad genetic base as quickly as possible. A practical application of collecting whitebark pine pollen will be supplemental mass pollination in the grafted seed orchard(s) to promote an earlier cone crop rather than relying on wind pollination. Unlike long-term storage of whitebark pine seed, there are no major pollen viability problems over the long-term with Pinus spp., as long as the pollen is properly extracted and stored.

Additional Gene Conservation Measures—Pollen will also be collected to establish a pollen bank as part of the ex situ strategy and to advance blister rust resistance in seed and breeding orchards.

The surviving progeny in each rust screening will be used to establish clone banks. Though not in our life times, these clone banks could serve as an operational cone collection site if they are designed by zone, concentrating the better performers in the interior core to enhance gain and in grouping trees by resistance mechanism, as is done in the Phase II western white pine seed orchards (Mahalovich and Eramian 1995).

Last, this information can be cross-referenced with field inventories to prioritize those communities that are good candidates to stabilize their numbers by active intervention involving prescribed fire to promote natural regeneration and by removal of encroaching species such as subalpine fir, lodgepole pine and Engelmann spruce.

Summary

This restoration strategy highlights the need to incorporate genetic considerations into a comprehensive strategy to restore whitebark pine. It emphasizes the biology and genealogy of the host species, with a modest emphasis on the biology and ecology of the rust. The amount of gain achieved in blister rust and mountain pine beetle resistance will be determined by how many cones are collected from presumably rust-free and insect-free trees in areas with a high frequency of blister rust and insect populations. Meaningful levels of genetic variation are needed in adaptive traits (for example, survival, growth, insect and disease resistance) to develop seed transfer guidelines and improved planting stock.

Acknowledgments

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