The Proactive Strategy for Sustaining Five-Needle Pine Populations: An Example of its Implementation in the Southern Rocky Mountains

Paper

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Abstract—The imminent invasion of the non-native fungus, *Cronartium ribicola* J.C. Fisch., that causes white pine blister rust (WPBR) and the current mountain pine beetle (*Dendroctonus ponderosae* Hopkins, MPB) epidemic in northern Colorado limber pine forests will severely affect the forest regeneration cycle necessary for functioning ecosystems. The slow growth and maturity of limber pine enables trees to persist on the landscape for centuries, but without sufficient regeneration opportunities these traits will inevitably hinder the ability of limber pine to adapt to novel stresses such as WPBR or climate change. The current MPB outbreak will result in the death of many mature limber pines, including many with genetic resistance to WPBR. It will be decades until advanced regeneration develops into seed-producing mature trees in much of this region. This development will be limited further by WPBR which rapidly kills susceptible young trees. Efforts to sustain limber pine and Rocky Mountain bristlecone pine in the southern Rockies were initiated in 2001. The Northern Colorado Limber Pine Conservation Program, described here, is an example of the Proactive Strategy being implemented at a local scale. The program is a cooperative partnership between the USFS Rocky Mountain Research Station, Rocky Mountain National Park (RMNP), USFS Forest Health Management, and several Ranger Districts on the Arapaho-Roosevelt National Forest. It is designed to 1) conserve limber pine genetic diversity and 2) collect needed data to assist land managers in making informed decisions for preparation of a management plan intended to sustain resilient limber pine ecosystems in northern Colorado. The cooperative effort aims to provide immediate protection of limber pine from MPB, facilitate seed collections for WPBR resistance tests, and store and use seed for conservation, research and restoration. Seedlings are being screened for WPBR resistance to determine frequencies of resistance across the landscape among populations and to identify resistant seed trees for future seed collections. The seedling tests will also estimate population differentiation along the elevation gradient to refine seed transfer guidelines. Surveys of forest health, biotic damage, rust incidence, and trends in advanced regeneration will help project persistence of these populations after passage of the MPB epidemic. These data will provide a basis for evaluations of proactive management options on a site specific basis before northern Colorado ecosystems are impaired by WPBR; this should shorten the time frame to return to functioning ecosystems. Focusing management on proactively maintaining genetic diversity and a functional regeneration cycle will promote sustained adaptive capacity and ecosystems resilience to novel stresses such as WPBR and climate change.

Introduction

There are still five-needle pine populations in North America that have not yet been invaded by *Cronartium ribicola* J.C. Fisch., the non-native, invasive pathogen that causes white pine blister rust (WPBR) (fig. 1). White pine blister rust will invade new areas where five-needle pines occur and will cause high mortality, potentially causing local extirpations. Options are available to land managers to help mitigate the impacts of rust and also to speed the recovery of the ecosystems. However, data is necessary to provide science-based information for developing those options. The Proactive Strategy framework is a template for the collection of this information in order to develop proactive management options. The proactive measures considered for WPBR will also assist with alleviating impacts of mountain pine beetle (*Dendroctonus ponderosae* Hopkins, MPB) and climate change. Any high elevation five-needle pine ecosystem not yet impacted by WPBR can be a candidate for proactive management.

Early awareness of the threat of WPBR to the remaining healthy high-elevation ecosystems offers unique opportunities for gathering information, capturing genetic resources, preparing the landscape for invasion, mitigating impacts of infection, and initiating preparations for restoration. Since high elevation ecosystems are especially vulnerable to climate change and mountain pine beetle activity has escalated recently in some of the five-needle pine ecosystems (Gibson and others 2008, Diaz and Eischeid 2007, Pepin and Lundquist 2008, Salzer et al. 2009)), both stressors add urgency and complexity to managing these mountain ecosystems. The Proactive Strategy was developed as a management tool to promote population and ecosystem resiliency in high-elevation five-needle pine ecosystems not yet infected by WPBR or in the early stages of infestation (Schoettle and Sniezko 2007; Keane and Schoettle this proceedings).

Recent discoveries of new WPBR infection centers in Colorado, Nevada and Arizona verify that the spread of the fungus continues (e.g. Blodgett and Sullivan 2004; Vogler and Charlet 2004; Kearns and Jacobi 2007, Fairweather and Geils 2011), although most five-needle pine populations

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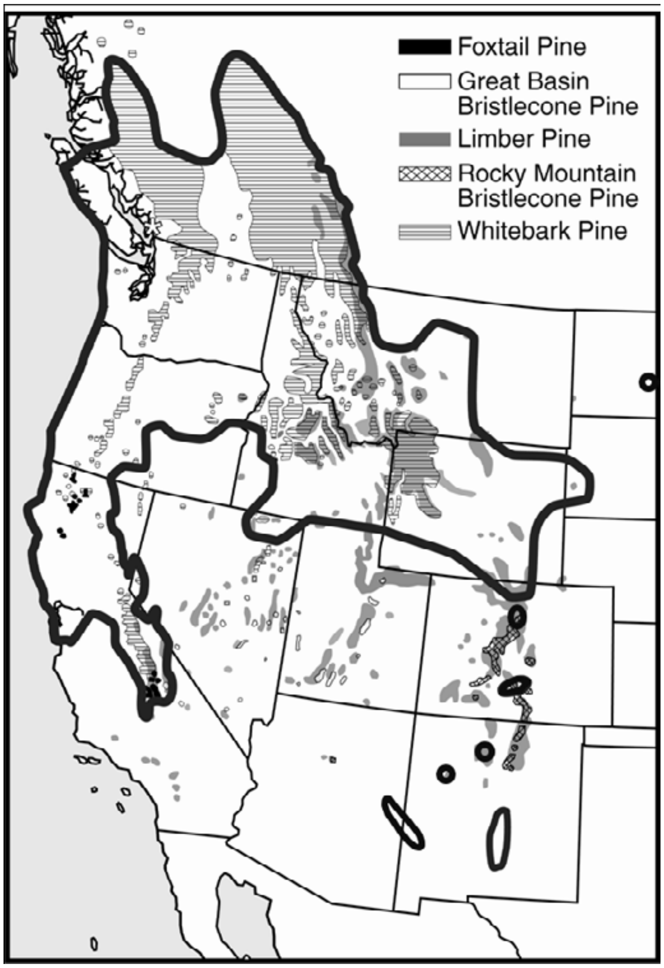
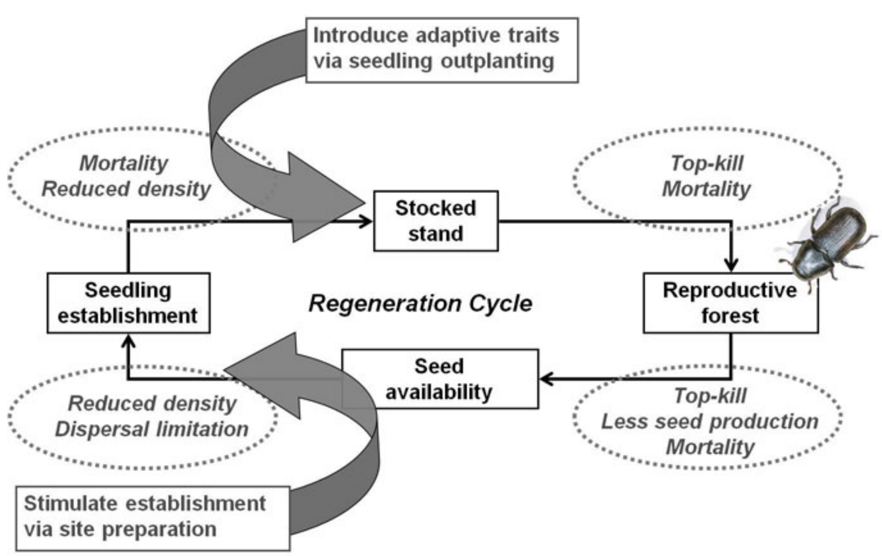


Figure 1. Map of western North America showing the current infection front of white pine blister rust (bold line; adapted from Samman and others 2003 and Schoettle and Sniezko 2007) and the distribution of each of the high elevation five-needle pine species. Note that many but not all populations within the infection areas are infected.

in these states are still healthy. Risk analyses of the currently rust-free areas of Colorado reveal that up to 50% of the five-needle pine habitats have climatic conditions that can regularly support disease success, suggesting that once the inoculum is in the area, disease incidence will increase (Kearns 2005; Howell and others 2006). The basic options for these areas include 1) 'no action': do nothing and let pine populations degrade and impacts cascade through the ecosystems, 2) 'reactive': wait to see if populations become degraded and then implement restoration treatments or 3) 'proactive': implement treatments before ecosystem degradation to maximize resiliency of the pine populations and mitigate ecosystems impacts. The detrimental impacts of WPBR in whitebark pine ecosystems (see Tomback and others 2001) demonstrate consequences of the 'no action' option in the early stages of infestation, an outcome which, in combination with the stress of MPB and climate change, has led to a petition for listing of whitebark pine as an endangered species under the Endangered Species Act (NRDC 2008).

Natural selection for traits that support survival under new conditions, such as an introduced pathogen or climate change, requires a foundation of genetic diversity and regeneration opportunities to pass the traits on to the next generation (Aitken and others 2008; St Clair and Howe 2009). Therefore, a sustainable population in the presence of WPBR requires traits for genetic rust resistance and a functioning regeneration cycle (Schoettle and Sniezko 2007). All of the high-elevation five-needle pines have at least one mechanism of resistance to WPBR (Hoff and others 1980). However, the frequency of trees with rust resistance appears to be low. These pines also have a slow regeneration cycle: they are not prolific seeders, they have delayed maturation and the adults are very long-lived. Proactive measures that enhance a functioning regeneration cycle in healthy populations can facilitate an increase in frequency of rust-resistant traits (Fig. 2). For example, artificial regeneration with rust-resistant seedling stock can increase the frequency of resistance in a healthy population that has very low resistance. If moderate frequencies of resistance exist in the healthy population, stimulating natural regeneration through site

Figure 2. Flow diagram of the forest regeneration cycle and the points of interaction with WPBR and management interventions. White pine blister rust can cause impacts at all stage (ovals) and the mountain pine beetle preferentially kills larger trees. Broad arrows depict intervention options for increasing rust resistance and population resiliency. (Redrawn from Schoettle and others 2009)



preparation results in greater numbers of individuals (and genotypes) on which natural selection for resistance can act (Schoettle and Sniezko 2007). These measures will be more effective at sustaining high mountain ecosystem services if implemented proactively in functioning ecosystems rather than reactively in degraded ecosystems where the regeneration cycle is impaired by WPBR. Many factors need to be weighed when making the decision to commit toward one or both of these proactive paths.

The Proactive Strategy provides a science-based foundation to better guide management decisions before ecosystem impacts curtail any options. This strategy, initiated in 2001 to sustain limber pine and Rocky Mountain bristlecone pine in the southern Rockies includes seed collections, forest health monitoring and studies of rust resistance, fire ecology, epidemiology, and regeneration dynamics (Schoettle 2004a; Schoettle and Sniezko 2007; Schoettle and others 2007; Burns and others 2010; Keane and Schoettle this proceedings, Kearns 2005; Kearns and Jacobi 2007). In this paper, we will present a summary of the intensive Northern Colorado Limber Pine Conservation Program as an example of the Proactive Strategy. The paper will provide guidance to land managers with concerns about the future viability of their five-needle pine populations. New science-based information is now available to help managers weigh some of the proactive management alternatives.

Urgency and Timing of the Proactive Strategy

Continued discoveries of WPBR infections in northern Colorado, including in Rocky Mountain National Park (RMNP) in 2009 and 2010, in conjunction with mountain pine beetle (MPB) populations increasing to epidemic levels heightens the concern over the health and future of limber pine in northern Colorado (Schoettle and others 2008; Schoettle and others 2009). The impact of these stressors together will severely affect pine regeneration cycles required for sustaining populations and ecosystem functions (Fig. 2). Although the slow growth and maturity of limber pine enable trees to persist on the landscape for centuries, the ability of limber pine to adapt to novel stresses, such as WPBR and climate change, will be hindered without sufficient regeneration opportunities. Moreover, the MPB outbreak will result in the death of many mature trees and, as a consequence, decades will pass until advanced regeneration can mature enough to produce seed. The ability to produce seed could take 30-50 years in limber pine. This recovery will be limited further by WPBR killing susceptible young trees as the pathogen expands its distribution.

WPBR can reduce the genetic diversity of pine populations (Kim and others 2003) and even cause population extirpation (Tomback and others 2001), so genetic conservation efforts should precede WPBR impacts. In light of the ongoing spread of WPBR and current MPB epidemic, capturing genetic diversity in the form of seed is an important step towards keeping proactive management options open into the future. The death of seed-producing mature trees by MPB will restrict our ability to capture the species' full genetic diversity and test for WPBR resistance. Current rust resistance testing technology requires the sowing of seed for artificial rust inoculation of seedlings. Non-reproductive young pine trees, which will escape MPB attack, cannot be screened for resistance until they mature; therefore without seed collections from the current overstory trees, gene conservation and tests of heritable WPBR resistance will be limited for the foreseeable future. Seed from base populations are also important for studies of genetic structure and seed transfer in order to develop restoration protocols, although technologies using genomics may ease that requirement in the future.

The Northern Colorado Limber Pine Conservation Program

In 2008, USFS Rocky Mountain Research Station (RMRS), Rocky Mountain National Park (RMNP), USFS Rocky Mountain Region Forest Health Management, and several National Forest districts initiated an intensive program to conserve limber pine and provide information for the development of limber pine management plans for Rocky Mountain National Park and northern Colorado; a process we are calling the Proactive Strategy. Approximately 1,100 ha and 41,000 ha of limber pine occurs in RMNP and Arapaho and Roosevelt National Forests, respectively. Most are in isolated populations east of the Continental Divide. Aside from the ecological functions that limber pine provides to high-elevation ecosystems including snow retention, wildlife food sources and facilitating the establishment of other tree species (see Schoettle 2004b), this long-lived species is aesthetically and historically important to visitors of the national park (2.9 million visitors in 2009; http://www.nps.gov/romo/parkmgmt/statistics.htm) and national forests. Limber pine occurs along scenic highways, along popular trails and at vista points that contribute to the visitor experience. Even people that do not visit these sites appreciate the high elevation pine species and value their continued existence (Meldrum and others this proceedings).

The objectives of the cooperative program to conserve and sustain limber pine on the northern Colorado landscape are 5-fold: (1) provide protection to limber pine from MPB so immediate seed collections can be made for WPBR resistance tests, genetic conservation, and research; (2) screen seedlings for WPBR resistance to determine the frequency of resistance across the landscape among populations and to identify resistant parent trees for future seed collections; (3) estimate population differentiation along the elevation gradient to refine seeds transfer guidelines; (4) survey forest health, biotic damage incidence and advanced regeneration to project persistence of these populations after MPB invasion; and (5) prepare management plans for northern Colorado.

1) Protection of Limber Pine from MPB for Seed Collections

Twenty-seven limber pine populations, stratified by elevation, were identified in RMNP and Arapaho and Roosevelt National Forest lands in spring 2008 (Figs. 3 and 4). Each population contains mature limber pine trees from which 10 to 15 individual seed trees were tagged and georeferenced for seed collections and MPB protection. Selected trees are spaced >60m from one another to increase sampling of non-related parent trees and the chances progeny will capture the pollen cloud of the population (and thus much of the genetic diversity); the sampling area per site ranged from approximately 9 to 12 ha.

Protection of limber pine seed trees from MPB on the 27 sites involves annual spraying with insecticides (at sites accessible by vehicle) or applying verbenone, a bark beetle anti-aggregate pheromone, on trees in more remote areas and within the National Park. Two pouches of verbenone are applied to each seed tree per year. Rocky Mountain National Park coordinates a network of citizen volunteers to help install the verbenone pouches at the park sites. This emergency short-term protection of the seed trees from MPB provides time to collect seeds during the epidemic.

Annual seed collections are attempted from each of the georeferenced seed trees at the 27 limber pine sites; bulk seed collections from other trees in each population are also being made (see Appendix for sampling details). Collections are made in late August through September and sometimes as late as early October, depending on seed maturation timelines. Generally, in northern Colorado, limber pine seeds mature at lower elevation sites in early September and at higher elevation sites by mid to late September. A bulk population collection from each site includes two to four cones from each of approximately 20 additional trees (not the georeferenced seed trees) evenly spaced throughout the population. Citizen volunteers also assist with cone collections each fall at the RMNP sites. Though 2008, 2009 and 2010 were not peak cone production years for limber pine in northern Colorado, collections were made from 201 individual seed-trees and an additional 23 bulk seed lots. Some of these were

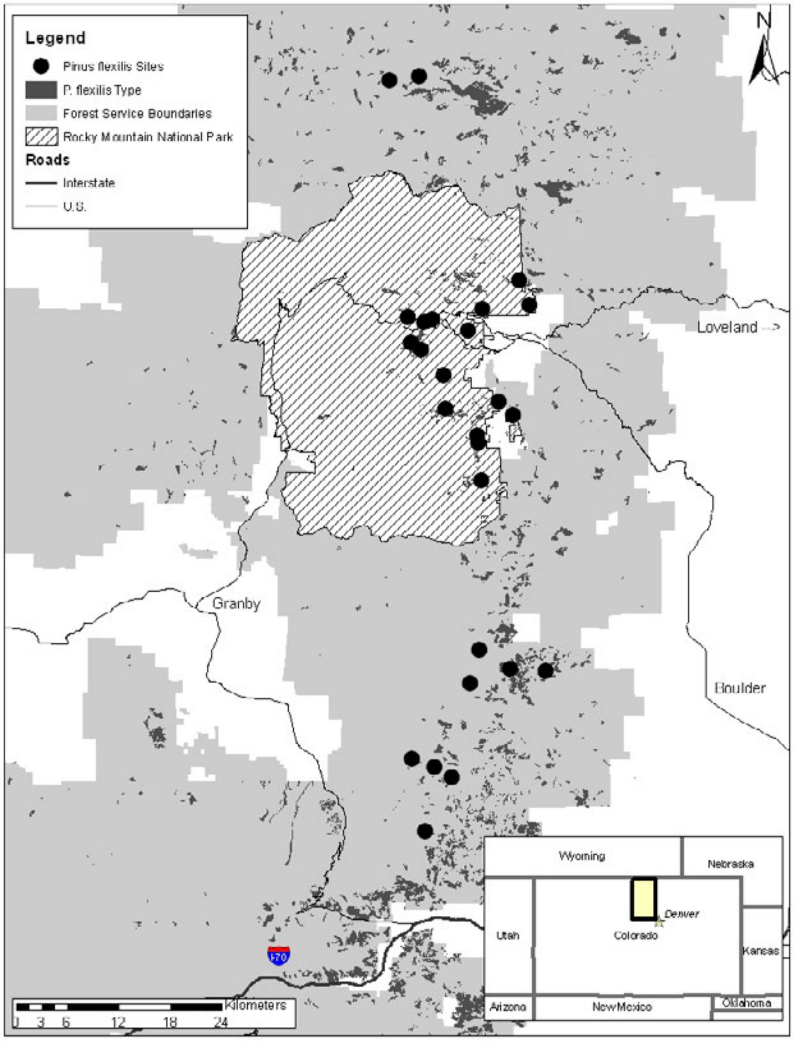


Figure 3. Map of the 27 sites used in the Northern Colorado Limber Pine Conservation Program.

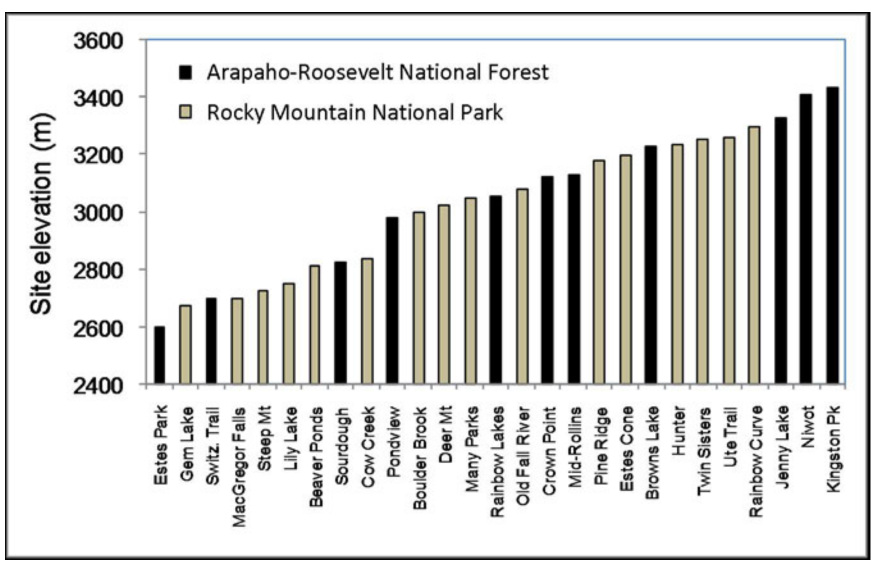


Figure 4. Distribution of the 27 sites used in the Northern Colorado Limber Pine Conservation Program with respect to elevation.

sampled in multiple years, bringing the total number of seed lots collected from the 27 sites in the past three years to 287. Seedlots with sufficient seed were used for rust resistance testing (see below) and a common garden study to assess variation among sources in growth parameters (see below). Remaining seed is archived at -80ºC for gene conservation, research and small scale outplanting projects.

2) White Pine Blister Rust Resistance Screening

Seed collections from bulk and individual tree collections are being tested for genetic resistance to WPBR at the Forest Service Dorena Genetic Resource Center (DGRC, Cottage Grove, OR). The initial tests are examining the incidence of canker-free seedlings ('complete' resistance) following artificial inoculation with rust (see Vogler and others 2006 for more information on complete resistance). Testing of the bulk seed lots will estimate the natural frequency of complete resistance in each of the national park and forest sites (populations) and seed lots from the individual seed-tree (family) collections will identify resistant trees that can be used as seed sources for future outplanting. Inoculation of seedlings from bulk seed lots was done in 2009 and from the individual seed-tree lots in 2010 using protocols developed for limber pine by RMRS and DGRC personnel in previous testing at DGRC. Disease development on seedlings will be tracked for two years post-inoculation and seed-tree (parent tree) resistance will be inferred from the proportion of canker-free progeny in each family. Earlier WPBR screenings have confirmed complete resistance in limber pine and refined the data collection methods to quantify disease development for this species (Kinloch and Dupper 2002; Schoettle and others in preparation). The stratification of sampling sites will help define the relationships between genetic resistance and elevation in this region, i.e., to explore whether negative correlations exist between rust resistance and other adaptive traits. The rust screening results will guide the design of additional bulk collections that will be needed to support future large-scale proactive or restorative measures.

3) Population Differentiation

Five seed zones have been defined for limber pine in the Interior West to guide seed transfer for artificial regeneration (Mahalovich 2006; Burns and others 2008). All of the limber pine populations in southeastern Wyoming, Colorado and northern New Mexico are in one zone with a recommended maximum elevational movement of seed of approximately 200 m. To refine this recommendation in northern Colorado, a common garden study of seedlings from the bulk seed lots is underway to assess population differentiation in growth traits. The results will help quantify adaptive traits of limber pine (i.e., differences in growth as well as in the timing of the growth period) from locations along the elevation gradient and refine seed transfer recommendations to reduce the possibility of maladaptation of future outplanting efforts.

4) Trends in Advanced Regeneration

Forest health and stand structure surveys were conducted in each of the 27 limber pine sites in the national park and surrounding national forest in order to estimate population resiliency to MPB-caused mortality. Surveys were conducted in 2009 during the initial escalation of the MPB epidemic in the limber pine type. Ten plots (0.02 ha each), spaced 50 m apart within each site were surveyed for site characteristics, disturbances, advanced regeneration, overstory density, tree health, crown class and biotic damages of all tree species (Klutsch and others this proceedings). Data collected specifically on limber pine included: seedling microsite, seed cone production, estimated tree ages, and annual shoot growth. Ground cover and canopy cover were also estimated in subplots. These data will provide projections of the persistence of these populations (as advanced regeneration) if all of the large reproductive limber pine are killed in the current MPB epidemic.

5) *Pulling It All Together and Building a Management Plan*

Protection of seed-trees will continue until the MPB epidemic subsides or further prioritization is possible given the results of the other components of the program. For example, the current resistance tests of seedlings from the individual-tree seed lots (i.e., families) will identify those seed-trees having heritable complete resistance; the seed-trees would then be a priority for continued MPB protection and seed collections for accumulating resistant seed stock to be used in future outplantings. The resistance being identified in these tests may be similar to complete resistance in western white pine and sugar pine (Kinloch and Dupper 2002). Selective pressure on the rust over time can lead to the rust overcoming at least one form of complete resistance in some pine species (Kinloch and others 1999, 2003, 2008). We do not know if this selection for virulence in the rust will occur in the limber pine-WPBR pathosystem in northern Colorado, but monitoring for symptoms of WPBR on the field trees having complete resistance (from the seedling rust resistance testing) will be useful for detecting changes in rust virulence over time. Known rust-susceptible seed-trees, as identified in rust resistance testing, may also be biological indicators of early WPBR presence at a site. Ultimately, we would like to know the frequency and distribution of all types of genetic resistance to the WPBR fungus. These tests are among the first for the species. The first prototype for the longer-term tests for limber pine was initiated in 2007 and could be extended to more parent trees pending additional funding. With further support and time, a more comprehensive look at the available array of resistances in these populations is possible and would be beneficial to building seed collections with more durable WPBR resistance and, ultimately then supporting future restoration projects. Knowledge of the type of resistance, their frequency and their geographic distribution will provide land managers with baseline data to augment their decisions on the need for or type of appropriate treatment for sustaining a population into the future.

Rust resistance data from the bulk seed collections will provide frequency estimates of complete resistance in each population. In addition, this resistance frequency may be present in all age classes of a population (i.e., not just in the mature trees of the population) because seed dispersal, in the absence of disturbance, is often local and rust is not yet putting selective pressure on the pine populations. The combined information on stand structure and bulk seedlot resistance data will give information about the likelihood that resistance traits will persist in the population, even if MPB kills the mature trees. For example, if a site has healthy advanced regeneration in limber pine and the resistance test of the bulk seedlot reveals resistance, we can suspect that the advanced regeneration also contain resistant genotypes. Therefore, the advanced regeneration at these sites may be prioritized for protection from post-MPB activities, e.g., dead tree removal or prescribed burning. In contrast, if advanced regeneration density is low at a site, yet the resistance test of the bulk seedlot reveals resistance at the site, then protection of the mature trees from further MPB impacts and site preparations to stimulate regeneration may be warranted. Furthermore, if a site has neither advanced regeneration nor any resistance, the common garden study can identify which of the other populations that did show resistance have similar survival and growth traits and can serve as seed sources for artificial regeneration of rust resistant seed or seedlings on the site. Likewise, enrichment plantings with seedlings from resistant parents can also be used in any site to increase the limber pine population and potentially increase local genetic variation. The results will be useful for designing additional bulk collections that will support future large-scale proactive or restoration measures.

Integration of the results from these data with results from other studies will provide the foundation for development of long-term management plans for limber pine in RMNP and northern Colorado. Data collection for these initial studies will be complete in 2013 when a management plan for limber pine will be prepared. Information about the genetic structure of these limber pine populations, persistence of populations after the MPB epidemic, rust resistance traits and current advanced regeneration trends will allow informed evaluations of proactive management options on a site-specific basis in northern Colorado. In addition, the sampling design described here will contribute information on the elevational distribution and coincidence of growth traits, stand structures and frequencies of resistance—information that may be relevant to other areas and possibly other pine-WPBR pathosystems.

Guidelines for successful outplanting techniques of limber pine seedlings in the Rocky Mountain region are under development (Casper and others, this proceedings) and will be applicable to northern Colorado should artificial regeneration be recommended. Furthermore, past research reveals that limber pine regenerates well after fire in this area (Coop and Schoettle 2009) indicating that the use of fire as a management tool may be an option to stimulate regeneration near populations that have resistance. Research on the colonization dynamics of limber in MPB-disturbed landscapes would also complement this program. Further investigation into the consequences of long-distance seed movement on tree performance, especially in light of possible climate change-WPBR interactions, is also recommended. Information from all of these studies would also contribute data to parameterize population or landscape models for limber pine, opening more opportunities to use modeling management tools. As more data and analyses are gathered (e.g., on rust resistance, regeneration following management treatments, etc.) management recommendations and activities can be modified accordingly.

Conclusions

The Northern Colorado Limber Pine Conservation Program is an example of implementing the Proactive Strategy to facilitate the development of a management plan. This program contributes site-specific scientific data and tools for decision-making about the need for and/or trade-offs of intervention to promote sustainability of northern Colorado limber pine populations in the presence of multiple stressors (WPBR and MPB). Some of the information, tools and activities, such as estimating rust resistance frequency, understanding natural regeneration dynamics, and capturing the pine's full genetic diversity via seed collections, can only be taken advantage of from a healthy forest that has not yet had its processes disrupted by these stressors. Likewise, some management options, such as stimulating regeneration in populations with high levels of rust resistance, are best implemented in a healthy forest. The early availability of information will facilitate the regulatory process if interventions are prescribed and permit the inclusion of science-based information in prioritizing sites for strategic planning. Gathering and using this information before the loss of ecosystem functions allows land managers the widest range of management options for maximum resiliency of limber pine, mitigating future impacts on these ecologically important ecosystems in northern Colorado.

This Proactive Strategy framework can be adapted for other five-needle pine species threatened by WPBR and/or MPB and possibly for other pathosystems. An intensive location-based program, such as that implemented in northern Colorado, is especially appropriate for administrative units that want to use local genotypes as much as possible, such as national parks. Understanding the interaction of the species' life history traits and ecology with resistance mechanisms will highlight factors that limit the species' sustainability in the presence of the pathogen. Focusing management on proactively maintaining genetic diversity and a functional regeneration cycle will promote sustained adaptive capacity and ecosystem resiliency.

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Appendix—Cone Collection Guidelines and Field Forms

Cone collection guidelines and field forms used for seed collection on *P. flexilis* and *P. aristata* in the southern Rocky Mountains and also for *P. flexilis* in Montana and for *P. longaeva* in Nevada. Collections are for gene conservation, rust resistance screening and research. These protocols have been adapted from those for *P. albicaulis* (Mahalovich personal communication).

This document outlines three tasks that should be conducted at each site: 1) 10 individual tree cone collections, 2) bulk cone collection, and 3) site characterization. Figure 5 is the cone collection field sheet and key; Figure 6 is a field data sheet. Additional information and photos are available upon request to Anna Schoettle (aschoettle@fs.fed.us).

Check cone and seed ripening:

Before collecting cones at a site, cut several cones on site and inspect the embryo for development. The embryo will be filling at least 90 percent of the cavity in the endosperm when the seeds are ripe enough to collect (Figure 7). The embryo should be matte in finish with no jelly-like texture.

Individual tree cone collection:

a. Find, tag (north side of tree) and GPS cone bearing trees, <u>each 200 ft away from each other</u>. If there is rust on site select 9 phenotypically resistant (no visible cankers) seed trees and 1 susceptible (cankered) seed tree. If there is no rust on site, select 10 healthy mature trees (minimize dwarf mistletoe [DM] presence).

<u>Spacing the trees:</u> The easiest way to space the trees is to GPS the first tree ("Main Menu → Mark → Waypoint"), then set your GPS unit to find that point (Find → Waypoint) and walk away from it until you are ~200 ft away and then start looking for the second tree and so on. Be careful if you are not moving in a straight line that you aren't doubling back and getting close to a former seed tree—you may need to do a "go to nearest waypoints" (Find → Waypoint) to make sure you are ~200 ft away from all your trees. Waypoints should be named the same as the TREE ID and marked as Site Initials (2 to 3 letters) and tag number in the GPS. Each seed tree should have a unique tag and ID number, even among sites. Example: UTE3426. Set GPS to Datum WGS84 under Settings/Setup → Units → Datum.

Cone collection information

This form has 2 pages --- a seedtree form and a site survey assessment form

(A)

Fill out one seedtree form for each site
- add extra sheet for more detailed site map if needed

Fill out one site survey assessment form per site

Questions? Contact Anna at (970) 498-1333

field	**Key**
SEEDTREE FORM	
Seed Zones	see map for your species
Crown Closure	open, closed, mixed
Tree species comp	list tree species present
Age structure	young, old AND uneven, even
Stand size	approximate acreage
MPB present?	MPB active in last 3 yrs = Yes; not active = No
WPBR present?	yes; no
5-ndle pine regen present?	seedlings or saplings that are less than 1.4m tall; rare, some, abundant
5-ndle pine regen health	good = regen has good new growth with no rust or DM fair = regen has good new growth but has rust or DM poor = regen is stunted and has little new growth and/or is diseased
other species regen present?	list other tree species with healthy vigorous regeneration
Height (m)	estimate tree height in meters
DBH (cm)	measure DBH in centimeters
Crown Position	dominant, subdominant
# bole cankers	number of WPBR cankers on the main stem of the tree
# branch cankers	estimate number of WPBR cankers on branches
# cones collected	record number of cones that are in the bag
SITE ASSESSMENT FORM	
walk a line (no formal transect) through the stand and record all trees greater than 1.4m	
species	4 letter tree species code (genus and species)
alive/dead	include only recent dead (<5 yrs with fine twigs present)
	A = alive; D = dead
dbh class	1 = less than 4 in dbh; 4 = greater than 4 in dbh
~ # cnkrs	estimate number of WPBR cankers
MPB	1 = hit by MPB; 0 = not hit
DM	1 = DM present; 0 = DM absent

Equipment you will need for one site:
Pole pruner (≥ 3 m)
GPS - loaded with gps points if seedtrees are already located
- check that datum is correct and recorded
extra batteries
hand clippers
bags (at least 11 bags - suggest 15-20)
tags to label and seal the cone bags
sharpies / pens / pencils
forms - seedtree form, 1 site assessment form, extra sheet for maps
metal tree tags (unique numbers)
Al nails
DBH tape (metric)
way to estimate tree in meters
camera - if possible - take a photo of tree looking north with person in photo with tree ID
area maps
latex gloves (for the cone bagger person - the cones are super sappy!)
vials with silica gel for needle samples (if collecting)
hand sanitizer gel (it removes sap!) and paper towels

Figure 5. Field cone collection key (A) and form (B).

(B)

Site Location and Collector Information					Stand Description			
Site Name and Initials (3 letters)			Collectors Names		Slope %	Aspect o from N	Tree species composition	
Seed Zone	Date of cone collections				Crown Closure		Age structure	Stand size (acres)
Preliminary tree selections made by:				Date of tree selections	MPB present?	WPBR present?		DM present?
National Forest		Ranger District		State	5-ndl regen present?	5-ndl regen health		Other regen present?
Township T_____	Range R_______	Section _______	Landowner	Datum	Other site landmarks, information			

Seed Tree Info

Species	Tree ID (site initials + tag#)	UTM Northing	UTM Easting	Elevation (m)	Height (m)	DBH (cm)	Crown Position	# bole cankers	# branch cankers	# cones collected	Other tree damages (DM, twig beetles), landmarks or comments
	-BULK	----------	----------	----------	----------	----------	----------	----------	----------		# trees sampled = ________; # cones/tree = __________

Directions to stand	Site map
On ______ side of road ______________________________	
miles from the junction with road ______________________________	
Other site descriptions / landmarks / former plot names	use additional sheet if necessary

Tree selection criteria: Healthy trees with cones! Single stemmed trees are preferred over multi-stemmed clumps. Multi-stem clumps can have multiple individuals and our "individual tree collections" must be from just one individual. If it is unavoidable to select a multi-stemmed clump then tag just one stem and be absolutely certain that all the cones are from just that stem. Note on form that tree is in a multi-stem clump. Make every effort to find single stem trees!

b. Record tree tag number, waypoint and associated information (ht, dbh, etc) as requested on the SEED TREE form (Fig. 5).

c. For each seed tree: Verify that the embryo fills at least 80 to 90 percent of the cavity in several seeds to determine if the seeds are ripe (Fig. 7). If ripe, collect at least 20-40 cones from as high in the crown as your pole pruner can reach. Try to collect only fully formed green cones (no small dry brown ones—they have aborted) from the upper third of the crown. Clip off the terminal branch with the cones, have the collector immediately retrieve the cones, detach cones from the twig and put only the cones in the burlap bag (fill each bag only half full—use 2 bags per seed tree if needed (see below). If you are not 100% sure that the cone you are picking up was cut from the sample tree do not include it (for the genetic analyses we have to be <u>absolutely certain</u> of the mother tree of the seeds).

Don't forget the bulk collection (see below)

d. Label both sections of the paper tag with the species, seed zone, elevation, Forest, District, collection date, collector name and TREE ID—detach lower portion and put it in the bag—use upper portion with a zip-tie to cinch the top of the burlap bag closed (within 6-8 inches of the top of the bag). Fill the bag only half full; we expect only 1 burlap bag per seed tree but if the cones are large and you need a second bag fill out a complete second tag and note on the tag that it is "1 of 2 bags" or "2 of 2 bags".

e. Fill out the field SEED TREE (Fig. 5) and SITE condition form (Fig. 6) for each site.

5-NEEDLE PINE CONE COLLECTION STAND SURVEY

Date of survey: ___________________

100 tree survey (trees greater than 1.4m tall) - 1 survey per site

AREA NAME

Tree ID tag #s at site:

	species	alive/dead	dbh class	~ # cnkrs	MPB	DM
1						
2						
3						
4						
5						
6						
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	species	alive/dead	dbh class	~ # cnkrs	MPB	DM
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52						
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Year of most recent MPB attack:

Estimate of years since WPBR invasion:

Figure 6. One-hundred tree forest health condition form

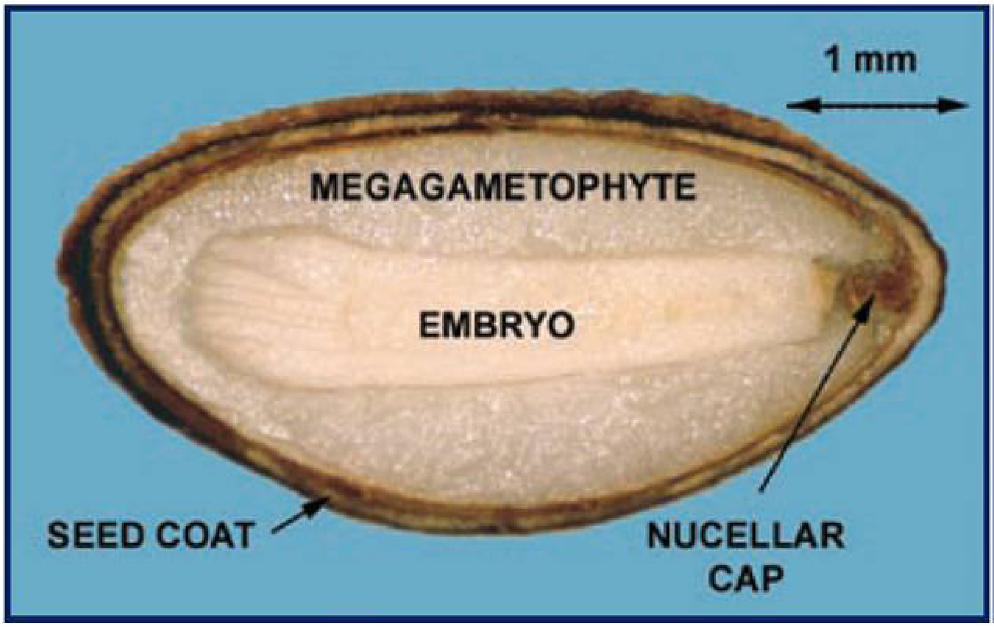

Figure 7. Photo of a healthy western white pine seed cut longitudinally revealing a fully mature embryo (from Kolotelo 1997 as presented in Owens 2004). The embryo fills the entire cavity in this photo. Seeds are mature enough for collection when the embryo is no less than 90 percent of the cavity. A healthy seed will have a bright white endosperm.

f. Keep the cones out of direct sun (especially in the back of a vehicle). Get them to a dry cool place with good ventilation between bags as soon as possible.

Bulk cone collection:

a. At each site, we want a bulk collection of cones that is a composite of equal number of cones from each of 20 trees (do not include trees with individual seed collections; select cones from high in the crown).

To do this, assess the amount of cone production in the site—if it is light then decide to collect 2 cones from each of the 20 trees; if it is a good cone producing site, select 4 cones from each of the 20 trees. The trees for the bulk need not be tagged or GPSed—they should however be somewhat evenly spaced throughout the population. The easiest way to do this is to collect from 2 trees for the bulk in between each individual seed tree you are collecting from. Keep track of the number of trees that contribute to the bulk sample (tally bulk tree numbers as you collect). Combine the 2 (or 4) cones from each tree into one burlap bag. Enter number of trees sampled and number of cones per tree on the SEED TREE FORM (Fig. 5).

b. Label the paper tag (upper and lower portions) with site name-BULK, "BULK from XXXX trees", GPS coordinates of the population, your name, date. As before, place the bottom half of the tag in the bag and use a zip tie to seal the burlap bag with the upper portion.

Site assessment:

It is important to have associated stand condition and forest health information at the time of the cone collections. For example, if a seed tree is recorded as being WPBR-free and the population has an estimated WPBR incidence of 60 percent, then that seed tree would be considered a putatively resistant tree. However, if that same tree was in a population with an estimated WPBR incidence of 5 percent it is much more likely to have escaped infection than be disease-free due to resistance. Any type of reputable stand assessment plot(s) can be used. If the site is co-located with a recent (past 2 yrs) stand health assessment then no further assessment is needed (unless the condition has significantly changed). Please provide a reference to the former data set, when it was collected and by whom, site name in the former study and a summary of the rust/MPB status of the site. At a minimum a 100 tree survey should be conducted.

100 tree survey: Walk a line through the population and record each tree (of all species) that is greater than 1.4 m tall onto the form (Fig. 6) along with the requested information. This does not need to be formal transect—try to cover the entire area within the population where the seed trees are located.

References

Aitken, Sally N.; Yeaman, Sam; Holliday, Jason A.; Wang, Tongli; Curtis-McLane, Sierra. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications. 1: 95-111.

Blodgett, James T.; Sullivan, Kelly F. 2004. First report of white pine blister rust on Rocky Mountain bristlecone pine. Plant Disease. 88: 311.

Burns, Kelly S.; Blodgett, James T.; Conklin, David; Fairweather, MaryLou; Geils, Brian; Guyon, John; Hoffman, James; Jackson, Marcus; Jacobi, William R.; Kearns, Holly S. J.; Schoettle, Anna W. 2010. White Pine Blister Rust in the Interior Mountain West. In: Adams, J. comp. 2010. Proceedings of the 57th Western International Forest Disease Work Conference; 2009 July 20-24; Durango, CO. Forest Health Technology Enterprise Team, Fort Collins, CO. Pp 57-65.

Burns, Kelly S.; Schoettle, Anna W.; Jacobi, William R.; Mahalovich, Mary F. 2008. Options for the management of white pine blister rust in the Rocky Mountain Region. Gen. Tech. Rep. RMRS-GTR-206. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 26 p. http://www.fs.fed.us/rm/pubs/rmrs_gtr206.html

Coop, Jonathon C.; Schoettle, Anna W. 2009. Regeneration of Rocky Mountain bristlecone pine (*Pinus aristata*) and limber pine (*Pinus flexilis*) three decades after stand-replacing fires. Forest Ecology and Management. 257: 893-903.

Diaz, H .F.; Eischeid, J. K. 2007. Disappearing "alpine tundra" Köppen climatic type in the western United States. Geophys. Res. Lett. 34: L18707.

Fairweather, M. L.; Giels, B. W. 2011. First report of the white pine blister rust pathogen, *Cronartium ribicola*, in Arizona. Plant Disease 'First Look' paper doi: 10.1094/PDIS-10-10-0699.

Gibson, Ken; Skov, Kjerstin; Kegley, Sandy; Jorgensen, Carl; Smith, Sheri; Witcosky, Jeff. 2008. Mountain pine beetle impacts in high elevation five needle pines: current trends and challenges. Forest Health Protection Report R1-08-020. U.S. Department of Agriculture, Forest Service. 32 p.

Hoff, Raymond; Bingham, Richard; MacDonald, Geral, I. 1980. Relative blister rust resistance of white pines. European Journal of Forest Pathology. 10: 307-316.

Howell, Brian; Burns, Kelly S.; Kearns, Holly S. J.; Witcosky, Jeff J.; Cross, Frank J. 2006. Biological Evaluation of a Model for Predicting Presence of White Pine Blister Rust in Colorado Based on Climatic Variable and Susceptible White Pine Species Distribution. Biological Evaluation R2-06-04, Rocky Mountain Region Renewable Resources, Lakewood, CO: U.S. Department of Agriculture, Forest Service. 15 p.

Kearns, Holly S. J. 2005. White pine blister rust in the central Rocky Mountains: Modeling current status and potential impacts. Fort Collins, CO: Colorado State University. 243 p. Dissertation.

Kearns, Holly S.J.; Jacobi, William R. 2007. The distribution and incidence of white pine blister rust in central and southeastern Wyoming and northern Colorado. Canadian Journal of Forest Research. 37: 462-472.

Kim, Mee-Sook; Brunsfeld, Steven J.; McDonald, Geral I.; Klopfenstein, Ned B. 2003. Effect of white pine blister rust (*Cronartium ribicola*) and rust-resistance breeding on genetic variation in western white pine (*Pinus monticola*). Theoretical and Applied Genetics. 106: 1004-1010.

Kinloch Bohun B., Jr.; Davis, Dean A.; Burton, Deems. 2008. Resistance and virulence interactions between two white pine species and blister rust in a 30-year field trial. Tree Genetics and Genomes. 4: 65-74.

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

Kinloch Bohun B., Jr.; Sniezko Richard A.; Barnes, Gerald D.; Greathouse, Tom E. 1999. A major gene for resistance to white pine blister rust in western white pine from the Western Cascade Range. Phytopathology. 89: 861-867.

Kinloch, Bohun B., Jr.; Sniezko, Richard A.; Dupper, Gayle E. 2003. Origin and distribution of Cr2, a gene for resistance to white pine blister rust in natural population of western white pine. Phytopathology. 93: 691-694.

Kolotelo, D. 1997. Anatomy and Morphology of Conifer Seed. B.C. Min. For., Nursery and Seed Operations Branch, For. Nurs. Tech. Series 1.1.

Mahalovich, Mary F. 2003. [Email correspondence to Anna Schoettle] June 4. Files included Cone collection guidelines. doc, WBP Survey Form rev 04-03.xls, WBP Location and GPS Record rev 05-2003.xls. On file at: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Colins, CO.

Mahalovich, Mary F. 2006. Limber pine seed transfer guidelines. U.S. Department of Agriculture Forest Service Regions 1-4. Internal Report, 4 pp. Prepared February 27, 2006.

Natural Resources Defense Council. 2008. A petition to list the whitebark pine, *Pinus albicaulis*, as an endangered species under the Endangered Species Act. December 8, 2008. 58 p.

Owens, J.N. 2004. The reproductive biology of western white pine. Forest Genetics Council of B.C. Extension Note 04, April 2004, p. 40.

Pepin N.C.; Lundquist J.D. 2008. Temperature trends at high elevations: patterns across the globe. Geophys. Res. Lett. 35: L14701.

Samman, Safiya; Schwandt, John W.; Wilson, Jill L. 2003. Managing for Healthy White Pine Ecosystems in the United States to Reduce the Impacts of White Pine Blister Rust. Forest Service Report R1-03-118. Missoula, MT: Department of Agriculture, Forest Service. 10 p.

Salzer, M. W.; Hughes, M. K.; Bunn, A. G.; Kipfmueller, K. F. 2009. Recent unprecedented tree-ring growth in bristlecone pine at the highest elevations and possible causes. P Natl Acad Sci USA. 106: 20348-20353.

Schoettle, Anna W. 2004a. Developing proactive management options to sustain bristlecone and limber pine ecosystems in the presence of a non-native pathogen. In: Shepperd, W. D., L. G. Eskew, compilers. Silviculture in special places: Proceedings of the National Silviculture Workshop; 2003 September 8-11; Granby, CO. Proceedings RMRS-P-34. Fort Collins, CO: U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station: 46-155. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p034/rmrs_p034_146_155.pdf

Schoettle, A. W. 2004b. Ecological roles of five-needle pines in Colorado: Potential consequences of their loss. In: Sniezko, Richard; S. Samman; S. Schlarbaum; H. Kriebel; eds. Breeding and genetic resources of five-needle pines: growth adaptability and pest resistance. 2001 July 24-25; Medford, OR. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 124-135. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p032/rmrs_p032_124_135.pdf

Schoettle, Anna W.; Rochelle, S. G. 2000. Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevation. American Journal of Botany. 87: 1797-1806.

Schoettle, Anna W.; Sniezko, Richard A. 2007. Proactive intervention to sustain high elevation pine ecosystems threatened by white pine blister rust. Journal of Forest Research. 12(5): 327-336.

Schoettle, Anna W.; Sniezko, Richard A.; Burns, Kelly S.; Freeman, Floyd. 2007. Preparing the landscape for invasion—Early intervention approaches for threatened high elevation white pine ecosystems. In: Goheen, E. M.; R. A. Sniezko, tech. coords. Proceedings of the conference Whitebark Pine: a Pacific Coast Perspective. 2006 Aug 27-31, Ashland, OR. R6-NR-FHP-2007-01. Portland, OR: Pacific Northwest Region, Forest Service, U.S. Department of Agriculture: 72-75. http://www.fs.fed.us/r6/nr/fid/wbpine/proc.shtml

Schoettle, Anna W.; Burns, Kelly S.; Costello, Sheryl; Witcosky, Jeff; Howell, Brian; Connor, Jeff. 2008. A race against beetles: Conservation of limber pine. Nutcracker Notes. 14: 11-12.

Schoettle, Anna W.; Sniezko, Richard A.; Burns, Kelly S. 2009. Sustaining *Pinus flexilis* ecosystems of the Southern Rocky Mountains (USA) in the presence of *Cronartium ribicola* and *Dendroctonus ponderosae* in a changing climate. In: Breeding and Genetic Resources of Five-Needle Pines Conference, IUFRO Working Party 2.02.15, Yangyang, Republic of Korea, 2008 September 22-26. Ed. By Noshad, D.; Noh, E.; King, J.; Sniezko, R. Yangyang: Korea Forest Research Institute: 63-65. http://www.iufro.org/download/file/4989/1191/20215-yangyang08-proceedings.pdf/

St. Clair, Bradley; Howe, Glen. 2009. Genetic options for adapting forests to climate change. Western Forester. 54(1): 9-11.

Tomback, Diana F.; Arno, Stephen F.; Keane, Robert E., editors. 2001. Whitebark Pine Communities: Ecology and Restoration. Island Press, Washington, DC.

Vogler, Det. R.; Charlet David A. 2004. First report of the white pine blister rust fungus (*Cronartium ribicola*) infecting whitebark pine (*Pinus albicaulis*) and *Ribes* spp. in the Jarbidge Mountains of northeastern Nevada. Plant Disease. 88: 772.

Vogler, D. R.; Delfino-Mix, A.; Schoettle, A. W. 2006. White pine blister rust in high-elevation white pines: screening for simply-inherited, hypersensitive resistance. In: Guyon, J. C., compiler. Proceedings of the 53th Western International Forest Disease Work Conference; 2005 September 26-30; Jackson, WY. Ogden, UT: USDA Forest Service, Intermountain Region. Pp 73-82.