Natural and anthropogenic threats to white pines from lower montane forests to subalpine woodlands of the Lake Tahoe Basin: An ecological and genetic assessment for conservation, monitoring, and management

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This proposal addresses two themes in the published RFP: 4-Managing the Basin’s Ecological Communities, and 6-Cross Cutting Science Areas (Sub-theme a).

**Justification:** Biotic (white pine blister rust, mountain pine beetle) and abiotic (climate, past logging, fire exclusion) stressors threaten white pines throughout their ranges in the Western U.S. At a landscape scale, the Lake Tahoe Basin Management Unit (LTBMU) can serve as a model to study natural and anthropogenic threats and their interactions on white pines from lower montane mixed-conifer forests (sugar pine, *Pinus lambertiana*) to upper montane forests (western white pine, *Pinus monticola*), to subalpine woodlands (whitebark pine, *Pinus albicaulis*). These species are key components of their forested communities, providing ecosystem services such as wildlife habitat, food resources, hydrologic functions, and biodiversity (Tomback et al. 2001 see chapters and references therein, Kinloch et al. 1996). The introduced pathogen *Cronartium ribicola*, cause of white pine blister rust (WPBR), is the greatest threat to white pine sustainability and survival. The disease can have negative effects on white pine population dynamics, genetic structure, and diversity (Tomback et al. 2001, Kinloch et al. 1996), influencing how these species respond to other stressors. A recent survey of WPBR on high-elevation white pines throughout California identified high rust incidence on whitebark and western white pine on the LTBMU and Eldorado NF.

Genetic resistance provides the best chance for survival of white pines under threat of WPBR. While simply-inherited mechanisms of resistance are well-documented in sugar and western white pines, little is known about resistance to WPBR in whitebark pine. Recent evaluations of resistance in Rocky Mtn. and Great Basin bristlecone pines—both, like whitebark, high-elevation species—reveal effective resistance in stems of some families (Vogler et al. 2006). Mechanisms that allow infection but prevent colonization are potentially valuable. This and other types of resistance may occur in whitebark pine, but data are limited. This study provides opportunity to test whitebark pine from established locations. Additionally, untested sugar and western white pine collections from lower and upper montane forests can be screened for resistance to WPBR.

Identifying changes in population dynamics of these white pines, as well as rust resistance, are critical to developing conservation and restoration strategies, given a variable and changing climate. Interactions among stressors must be considered: not only effects of WPBR, but effects of climatic change as it interacts with WPBR and mountain pine beetle (MPB) activity, and how these interactions will affect population and genetic structure. Established plots can be the foundation of long-term monitoring within these 3 forest types to document population, disease, and genetic dynamics for these focal species, as well as forest community responses to climatic changes. Developing a seed-bank of genetically diverse material for the LTBMU, including seed from rust-resistant parents, can be a model for genetic conservation in other National Forests and Parks.
Background/Goals/Objectives: California is rich in its white pine diversity, with six of the nine North American species distributed throughout montane ecosystems in the State. Three of those are key tree species in the montane forest communities of the LTBMU. The five important threats to these white pine species throughout their ranges and in the LTBMU are the introduced pathogen WPBR, mountain pine beetle (*Dendroctonus ponderosae*), climatic warming, past logging, and fire exclusion (see Table 1).

**White Pine Blister Rust.** WPBR is one of the greatest threats to white pines throughout their ranges and has caused widespread mortality and devastation (Schwandt 2006, Samman et al. 2003, Tomback et al. 2001). This invasive pathogen may also affect white pine population dynamics and genetic structure and diversity. The upper montane (western white pine) and subalpine (whitebark pine) forests of the LTBMU are the most impacted by WPBR. A study on WPBR in the Basin and a State-wide survey by R-5 Forest Health Protection identified high levels of WPBR on whitebark (mean 40%) and western white pine (mean 30%) in the LTBMU and Eldorado NF (Maloney 2000, Maloney et al. in prep). WPBR is causing demographic effects on white pine populations in California montane forests and in other locations in the West (van Mantgem et al. 2004, Ettl and Cottone 2002, Tomback et al. 2001, Maloney et al. in prep). Demographic effects include juvenile and adult mortality, lowered recruitment and reproductive output due to infection, and mortality of cone-bearing branches. Additionally, branch mortality can negatively affect gene flow (reduced wind pollination and seed dispersal).

**Mountain Pine Beetle.** MPB is a native insect present throughout montane forests in the Sierra Nevada. Its activity is often triggered by protracted droughts and by fire damage (see CFPC reports 1970 - 2006). Many parts of the West have and continue to experience devastating infestations of MPB. Future projections of MPB activity are alarming, with predictions of more severe and protracted drought periods given global climatic changes (Logan and Powell 2001). We see moderate levels of MPB activity but low levels of MPB-mediated mortality in the LTBMU. However, some of the highest observed mortality in the Basin is in WPBR-infected stands affected by drought stress (Maloney et al., unpublished). MPB activity can also be triggered when white pines have been damaged by fire, particularly sugar pine in mixed-conifer forests undergoing fire reintroduction (Schwilk et al. 2006, Maloney et al. in prep). In California, little is known about the biology and behavior of MPB in high-elevation forests (Sheri Smith, pers. comm.). Mortality occurs in lodgepole and mixed-white pine forests, but vast areas of dieback, with some exceptions, do not normally occur in California (see CFPC 2006).

**Climate.** Studies show clear evidence that climatic conditions are changing as a result of both natural and anthropogenic causes (IPCC 2001). Development of plant-climate models and paleoecological studies has broadened our thinking on how species may respond to changing climatic conditions (Rehfeldt et al. 2006, Davis and Shaw 2001, Jackson and Overpeck 2000). Bioclimatic models have been developed for the white pines in Western North America (Warwell et al. unpublished data), and conditions suitable for whitebark pine may decline dramatically in the Tahoe Basin by the year 2030, with losses of suitable habitat for western white pine and possible expansion of sugar pine at higher elevations (Fig. 1). While these models are based on a number of
assumptions (see Rehfeldt et al. 2006), they do not include microclimate, climatic variability, dispersal, population, genetic, or disease resistance data. Millar et al. (2004) caution that species shifts may not be linear (solely elevational or latitudinal) and shifts may occur due to differential topographical conditions and structural shifts. Bioclimatic predictions can be useful tools to identify populations at risk, as well as guide how we study and manage these species in light of anthropogenic impacts such as WPBR and future changes in environmental conditions in the LTBMU.

Past Logging. Extensive logging of the LTBMU during the Comstock era dramatically changed the composition and structure of these forests (Lindström et al. 2000, Manley et al. 2000). In some locations, sugar pine formerly comprised 20-25% of the forest, while at present it is only 1-6% (Barbour et al. 2002, Lindström et al. 2000 and references therein). Reforestation was not part of management during the Comstock era, so cutting of sugar pine affected not only population structure and dynamics but also genetic structure and diversity, with population and genetic losses. Such losses may affect sugar pine’s resilience to disturbances (e.g., WPBR, MPB, climate).

Fire exclusion. Fire exclusion policies have affected many forest types and species with subsequent increases in tree densities and fuel loadings (Sugihara et al. 2006). In the Sierra Nevada, much research has been done on the effects of fire and fire exclusion in montane forests (van Wagendonk and Fites-Kaufman 2006). van Mantgjem et al. (2004) and Maloney (2000) have found that mortality of sugar pine was often attributed to WPBR and competition-induced stress in fire suppressed mixed-conifer forests. In addition, forest restoration treatments and subsequent fire damage can increase bark beetle-mediated mortality of juvenile and reproductive sugar pines (Schwilk et al. 2006, Maloney et al. in prep). Such treatments may require special prescriptions to minimize loss of these valuable and reproductive trees, especially given the abovementioned losses from past logging.

Given these threats, an ecological and genetic assessment is warranted for developing conservation and management strategies for these species, given a changed and changing environment. Our objectives are:

1. Establish a network of ecological monitoring plots in lower montane mixed-conifer forests (focal species-sugar pine), upper montane forests (focal species-western white pine), and subalpine woodlands (focal species-whitebark pine) in the LTBMU to identify population structure and evaluate stressors associated with each of the 3 focal species.
2. Identify population genetic structure and diversity, and WPBR-resistance frequency, for populations of each focal species.
3. Develop demographic models to determine population dynamics.
4. Develop and test the efficacy of conservation and restoration strategies.

With the information gathered we can develop conservation and restoration treatments for populations that show impacts on genetics (low diversity, little or no rust resistance) and population dynamics (declining due to lowered recruitment and reproduction).
Geographic Location/Approach/Methodology:

Geographic location. Research will cover much of the LTBMU, including parts of the Eldorado NF, Desolation Wilderness, and the Toiyabe NF. This area also includes protected lands in California and Nevada State Parks. All lab work, genetic analyses, and whitebark resistance evaluations will be done at IFG and NFGEL, Placerville, CA. Cone processing, seed banking, and resistance screening of western white and sugar pine will occur at the Placerville Nursery.

Objective 1. Establish a network of ecological monitoring plots in lower montane mixed-conifer forests (focus: sugar pine), upper montane forests (focus: western white pine), and subalpine woodlands (focus: whitebark pine) in the LTBMU to identify population structure and evaluate stressors associated with each of the 3 focal species.

Thirteen transects will be established in the Basin, with populations sampled in each of the elevational forest types, where present, for sugar pine (11 populations), western white pine (13 populations), and whitebark pine (10 populations). One transect will be located in each of the quadrangles of the LTBMU to capture the different physiographic regimes in the Basin. Within each population, 3 replicated (0.5 - 1.0 ha) plots will be established for sampling, mapping and long-term monitoring. Within plots, tree, environmental, disease (WPBR), MPB, other biotic and pest, and land-use history data will be collected. PRISM (climate) data will also be generated for each plot location. Trees will be tagged at breast height (1.37 m) so that subsequent evaluations can be accurately measured for growth. Mortality and causes of mortality will be assessed and approximate year of death will be estimated. Recruitment (seedlings and saplings < 1.37 m at breast height) will be evaluated within each plot with 3 nested regeneration plots (15 m x 15 m), for a total of 9 permanent regeneration plots/population. All regeneration will be tagged, species identified, condition evaluated, and whorls counted for aging (a sample of out-of-plot trees will be cored to confirm age with whorl counts from similar-sized trees within regeneration plots). Reproductive output will be assessed by counting cones from 10 WPBR-infected and 10 uninfected trees per plot (for a total of 30 infected trees and 30 uninfected trees/population). To separate the effects of climate and WPBR on recruitment, an ANOVA and time-series analysis will be employed. Within each of the plots a forest vegetation plot (40 m x 40 m) will be established to assess forest composition and structure. Similar data will be taken as above for all tree species as well as shrub-cover and focal and non-focal species recruitment data.

MPB traps will be set-up in 3 populations for each white pine species to quantify numbers of MPB associated in each of the elevational forest types, and to be correlated with biological and environmental data. WPBR cankers will be aged to determine infection years and climatic conditions associated with those infection years. Cross sections will be taken through the canker center and at the base of the stem or branch to provide an unaffected baseline for tree ring comparisons (Kearns 2005). Cross-dating techniques (Stokes and Smiley 1968) will be used based on chronologies from the sample sites to determine year of infection. Because lag periods exist between initial infection
and subsequent canker formation, climate data will be correlated with year from cross-dating information as well as 1–4 years prior.

**Objective 2. Identify population genetic structure and diversity, and WPBR-resistance frequency, for populations of each focal species.**

**Population genetic structure.** Genetic information is essential to understanding ecosystem and species integrity. Issues concerning genetic structure and diversity, inbreeding, gene flow, and adaptation must be understood to ensure successful restoration. Isozyme analysis has been widely used over the decades to study genetics of many organisms. There is a rich body of information characterizing isozyme variation in diverse plant species (Hamrick and Godt 1990), and there is standard methodology easily applied to all species (Soltis and Soltis 1989, Conkle et al. 1982). Isozyme analyses provide codominant markers in numbers sufficient to address many scientific questions (Avise 1994, Soltis and Soltis 1989). Because isozymes reveal only a fraction of the variation present in genomes, other methods are used with isozymes to provide genetically more variable markers, including SSR (Simple Sequence Repeats). SSR markers are attractive for use in population genetics because they are typically co-dominant, multiallelic, highly polymorphic, abundant, and uniformly dispersed throughout the genome, and efficiently analyzed by a fast and simple PCR assay (Powell et al. 1996).

For this study, we will collect both seed and foliage for genetic analyses via Laboratory Standard Operating Procedures (SOPs) (USDA–FS 2005). With these collections we can assess genetic structure of adults and recruitment and diversity of these populations, and interpret such diversity in relation to ecological stressors and evolution. Conifer tissue will be ground to extract water-soluble proteins as well as genomic DNA (SOPs as in USDA–FS 2005). Isozyme markers will be analyzed using GDA, POPGENE, and GENALEX software packages. Genomic DNA data will be analyzed using, at a minimum, MICROSAAT and GENALEX.

**Mechanisms and heritability of WPBR.** Since the 1970’s, we have known of a major gene for resistance (MGR) in sugar pine that confers immunity against WPBR (Kinloch et al. 1970). MGR operates in needles, where hosts respond to infection by producing a hypersensitive spot that prevents fungal growth (Kinloch and Littlefield 1976). Infection proceeds normally in trees without this gene, and hosts are eventually deformed and killed. Another MGR-type gene was found in western white pine (Kinloch et al. 1999). These genes are the foundation for resistance to WPBR in California, and the basis for operational screening of white pine seedlings at the Placerville Nursery. The screening protocols are standardized and efficient and will provide an overview of the frequency of MGR in LTBMU populations.

In whitebark pine, no MGR has yet been found. Screening at the Dorena Center in Oregon shows that some whitebark families are highly-resistant to infection, but the mechanism and inheritance are unclear (R. Sniezko, pers. comm.). Research in Rocky Mtn. and Great Basin bristlecone indicates a resistance mechanism whereby WPBR
grows normally through needles (no MGR), but fails to infect stems (Vogler et al. 2006). Such mechanisms may occur in whitebark.

At IFG, we propose to test whitebark from five LTBMU populations (30 parents each from two populations, and 20 parents each from three populations) using standard inoculation protocols (Kinloch et al. 1999). Seedlings will be sown and cultured for 1 season (cotyledon stage), 2 seasons (primary needle stage), and 3 seasons (secondary needle stage). Seedlings of each age will be inoculated in Percival™ dew chambers with WPBR inoculum. Inoculating at different developmental stages is crucial for high-elevation white pines, since they grow more vigorously in greenhouse culture and can die before being adequately tested for resistance to WPBR. Seedlings will be inoculated and observed using standard protocols (Kinloch et al. 1999). Resistance expresses slowly in high-elevation white pines, hence each set will be observed for at least 3 years post-inoculation.

**Objective 3. Develop demographic population models to determine population dynamics.**

Ecological data from Objective 1 and resistance and genetic data from Objective 2 will be used to develop matrix models to assess population dynamics and determine if these populations of white pine species are, declining ($\lambda < 1$), maintaining ($\lambda = 1$), or growing ($\lambda > 1$) in the LTBMU. For details on this approach, see Caswell (2001). Censused individuals from population (plot) data will be placed into size-based stages to construct Lefkovitch (1965) transition matrices. These matrix models will then be used to project the size and structure of white pine populations in time. Elasticity analyses will be calculated to determine the relative contribution of demographic parameters (matrix element) to population growth rate ($\lambda$). We will assess the response of population growth, $\lambda$, to the effects of disturbances such as a WPBR wave year (which can affect reproductive individuals and subsequent recruitment, as well as mortality). Incorporating levels of resistance into the matrix models may allow for a more accurate estimation of population growth and dynamics. Inclusion of genetic diversity measures will be explored. Such demographic models have been employed for white pine species to assess population dynamics in WPBR-impacted whitebark pine populations in Mt Rainier NP (Ettl and Cottone 2002) and in sugar pine populations affected by WPBR and fire exclusion in Yosemite and Sequoia and Kings Canyon NP (van Mantgem et al. 2004). Use of a demographic approach will allow us to identify the status of these white pine populations (healthy or declining). If populations are showing signs of decline, as a result of WPBR or past logging, for example, we can identify where in the life cycle is the appropriate target for restoration and management.

**Objective 4. Develop and test efficacy of conservation and restoration strategies.**

In WPBR-affected stands or where populations have been reduced by logging or fire, restoration may be required. Reforestation requires stock that is both locally-adapted and WPBR-resistant; without resistance, restoration will likely fail. We propose to test two methods of deploying resistant and locally-adapted white pine material.
1) Direct sow seed. We will direct-sow in and adjacent to established populations, using cages to protect buried seed from predation (Schwandt 2006). Planting locations will be selected from populations showing a decline.

2) Plant seedlings. Protocols for planting western white and sugar pine are well established. For whitebark, we will experiment with greenhouse seedlings grown to a range of sizes.

**Deliverables/Products**

This proposal will deliver several significant and previously unavailable products regarding white pines in the LTBMU: genetic structure and diversity of populations; information on severity of natural and anthropogenic threats; data on mechanism and frequency of WPBR-resistance; a seed-bank of local and potentially rust-resistant seed; species and site-specific demographic models; identity of WPBR wave years; recruitment dynamics for each species; long-term ecological monitoring plots; and, testing of practical restoration treatments.

**Schedule of Events**

Year 1 – All plot work (outlined in Objective 1) will be done in this year as well as tissue collections for adult tree genetic analyses. Cones will be collected from whitebark pine and sugar pine (for seed-banking) and whitebark pine resistance mechanism and inheritance research will begin at IFG; sugar pine will be screened for resistance. Develop size/stage-structured population matrices.

Year 2 – Collect next generation (recruitment) tissue for genetic analyses. Establish and test restoration treatments (seed versus seedlings) in each forest type (where population status has been identified). Western white pine cones will be collected for seed-banking and resistance screening and whitebark pine resistance evaluations continue. Further develop demographic models with available genetic data.

Year 3 - Continue with whitebark pine resistance evaluations and model development. Evaluate seed and seedling restoration treatments.
References


Maloney, P.E., T. F. Smith, C.E. Jensen, D.M. Rizzo, J. Innes, and M.P. North. in preparation. Tree mortality and pest responses to fire and thinning in a mixed-conifer ecosystem of the Sierra Nevada, California, USA.


Table 1. Current stressors/threats to five-needled white pines species in the LTBMU.

<table>
<thead>
<tr>
<th>Stressors/threats</th>
<th>Sugar Pine Lower montane mixed-conifer</th>
<th>Western White Pine Upper montane forests</th>
<th>Whitebark Pine Subalpine forests</th>
</tr>
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<tbody>
<tr>
<td>Invasive pathogen-WPBR</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>MPB</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Climate</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Past logging</td>
<td>High</td>
<td>Low-moderate</td>
<td>Low</td>
</tr>
<tr>
<td>Fire exclusion – fire restoration prescriptions</td>
<td>High</td>
<td>Low-Moderate</td>
<td>Low</td>
</tr>
</tbody>
</table>
Figure 1. Bioclimatic models and projections of white pine species distributions in the LTBMU vicinity; present day and 2030. Color index at left corresponds to number of votes (model generated) for species presence within a 1 km pixel, given the climatic conditions at that location.

Votes for species presence
- 90 - 100
- 80 - 89
- 70 - 79
- 60 - 69
- 50 - 59

<table>
<thead>
<tr>
<th>Present</th>
<th>2030</th>
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</thead>
<tbody>
<tr>
<td>Subalpine forests - Whitebark pine</td>
<td></td>
</tr>
<tr>
<td>Upper montane forests - Western white pine</td>
<td></td>
</tr>
<tr>
<td>Lower montane mixed-conifer forests - Sugar pine</td>
<td></td>
</tr>
</tbody>
</table>

Bioclimatic models adapted from and courtesy of M.V. Warwell, G.E. Rehfeldt, and N.L. Crookston (unpublished data); for detailed descriptions of model development see Rehfeldt et al. 2006.