

cultural regimes for growing whitebark pine at dorena genetic resource center

lee riley, carmen coumas, jodie morrison, judith danielson, & richard sniezko
usda forest service, umpqua national forest, dorena genetic resource center, cottage grove oregon



grizzly bear fecundity is directly related to a diet of whitebark pine cones



maturing whitebark pine cones



Clark's nutcracker is the main dispersal agent for whitebark pine



germinating whitebark pine seed



natural whitebark pine seedling clump



Ribes leaf infected with blister rust spores



whitebark pine sapling infected with blister rust



OBJECTIVES

In order to produce whitebark seedlings in nursery regimes, for both blister rust screening and reforestation, some inherent problems of regeneration in the species must be addressed. Seed germination is generally both poor and erratic; only 10 to 15% germinate in the first year under natural conditions. The presumed reasons for this include: predation (and caching) of seed crops before embryo maturity by various animals, lack of appropriate substrate and climatic conditions, complex dormancy-release physiological requirements, and extremely hard seed coats. All of these factors can be positively adaptive in a natural environment over a period of time, providing a small supply of germinants over a period of 2-3 years given the proper conditions. But they present serious challenges in a nursery environment. The previous literature on attempts to successfully produce whitebark seedlings is varied but not extensive, and determining a routinely satisfactory protocol has been difficult. The most successful procedures to date are those developed by Coeur d'Alene Nursery (Burr and others 2001). In this study, the objective is to compare Coeur d'Alene Nursery's protocol with a variety of pre-sowing and culturing treatments to determine proper growing procedures for this difficult species in a different growing environment and with seed lots of various ages and from different provenances.

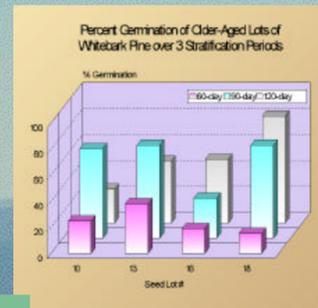
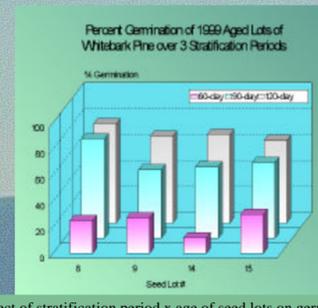
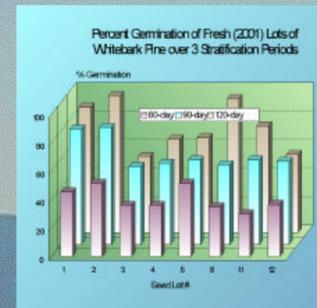
INTRODUCTION

Whitebark pine (*Pinus albicaulis*) is a slow-growing, wide-ranging conifer species of high elevation ecosystems in western North America, disjunctly distributed in timberline habitats of two mountain sequences: the first including the British Columbia Coast Ranges, the U.S. Cascade Range, and the Sierra Nevada, and the second covering the Rocky Mountains from Alberta to Wyoming; with outliers into Nevada and Utah. It is the only North American member of the stone pine group (*Cembrae*).

Whitebark pine is of little commercial interest, but is important in watershed stability and high country esthetics. Its seeds are a primary food source for red squirrels, grizzly and black bears, and nutcrackers, as well as others. There is evidence to support a theory of mutualist co-evolution with the nutcracker, on which the tree depends for natural seed dispersal, site selection, gene flow, and regeneration. Other evidence implicates the periodicity of whitebark seed production in the reproductive success of bears.

In some areas of its range, whitebark pine populations have decreased precipitously within the past 20 years. Three major causes of this decline are presumed: successional replacement by other seral species due to fire suppression policies, epidemics of bark beetles migrating from other pine species in lower elevation ecosystems, and the expanding range of the exotic fungal disease white pine blister rust, caused by *Cronartium ribicola*. Whitebark pine is generally regarded to be one of the 5-needed pine species most susceptible to blister rust attack and mortality.

Various plans to aid in management and regeneration of the species have been formulated; broadly, the two main approaches include silvicultural treatments with fire, and artificial regeneration and screening for blister rust resistance, with survivors used to regenerate stands. To this end, Dorena Genetic Resource Center is initiating a program of germinating and culturing whitebark pine seedlings for both short term and long term blister rust resistance screening.



Effect of stratification period x age of seed lots on germination



MATERIALS AND METHODS

Twenty lots of whitebark pine (WBP) were used in this study, ranging in storage time from 0 to 7 years. Nineteen lots were individual tree collections from 5 forests in Washington and Oregon. One was a bulk seed lot from Region 1. Filled-seed % ranged from 18-100. Prior to use, seeds from each lot were X-rayed on a template to extract only filled seed and assess for embryo quality. A total of 504 filled seeds from each lot were pulled and divided into a total of 24 treatments (21 seeds/treatment). Experimental design consists of four fixed main effects that are crossed: pretreatment, stratification time, germination temperature, and culturing regime. The GLM Mixed Model ANOVA was utilized on % germination, with the following transformation: arcsin $\sqrt{(\% \text{germ}/100)}$.

Seed pretreatment

Treatment SP-1. Control treatment as utilized by Coeur d'Alene Nursery (Burr and others 2001). Seed from each lot was placed in labeled mesh bags and soaked for 48 hours in running tap water to remain aerated during water absorption.

Treatment SP-2. Standard operating procedure for seed pretreatment at Dorena GRC. Seed from each lot was placed in labeled mesh bags and soaked for 24 hours in a 1% H₂O₂ solution. At the end of 24 hours, seed was rinsed and then soaked for an additional 24 hours in water.

Stratification time

All seed was subjected to a 30 day warm stratification period - i.e. placed in germinators and maintained at 20°C night/22°C day (68°/72°F) (Burr and other 2001). Three different cold stratification treatments (all 1-2°C (34-36°F)) followed the warm stratification period.

Treatment ST-1. 30 days cold (60 days total)

Treatment ST-2. 60 days cold (90 days total) (control)

Treatment ST-3. 90 days cold (120 days total)

Germination

At the conclusion of all stratification periods, each seed not showing an obvious split was nicked with a razor blade along the main line dividing the 2 halves of the seed coat. All nicked seed were placed on moistened blotter paper in 4"x4" clear plastic boxes.

Treatment G-1. Control treatment as utilized by Coeur d'Alene Nursery (Burr and others 2001). Boxes were placed in a germinator maintained at 20°C night/22°C day (68°/72°F) with a 12 hour photoperiod.

Treatment G-2. Boxes were placed in a germinator maintained at 16°C night/18°C day (61°/64°F) with a 12 hour photoperiod.

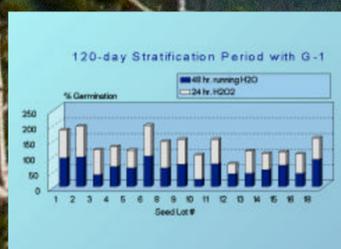
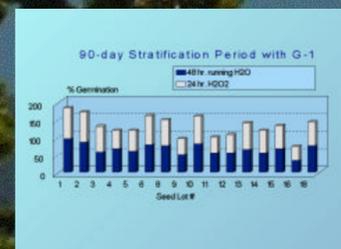
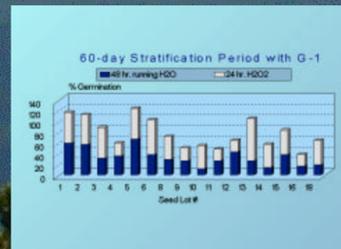
All lots were monitored for germination every 48 hours. Seeds were considered germinated when the radicle protruded from the seedcoat to a length of 2 mm and curved. These germinated seeds were removed from the germination boxes, planted into supercells containing pre-fertilized and pre-moistened "seedling mix", and covered with nursery grit. Following planting, supercells were placed in racks and kept in a somewhat climate controlled room at approximately 21°C (70°F). Assessment continued for 5 weeks.

Culturing

At the end of the 7 days in the climate controlled room, cells were moved to a greenhouse environment. Two different greenhouse environments are currently being tested during this study.

Treatment C-1. Seedlings are currently being grown in a new climate controlled greenhouse, with daytime temperatures maintained at or below 21°C (70°F), covered with Klerk's KoolFilm®.

Treatment C-2. Seedlings are currently being grown in the standard greenhouse growing area, which is subject to ambient conditions for low-elevation western Oregon.



Effect of stratification period and seed pre-treatment on germination under one temperature regime

PRELIMINARY RESULTS

- Seed lots were significantly different for % germination (p<0.0001). Seed pretreatments were significantly different (p=0.01) but varied depending upon the length of the stratification period (p<0.0001) and the seed lot (p=0.002).
- Due to the interaction between seed pretreatment and stratification period, no conclusions can be made regarding these main factors. Both main effects vary depending upon the seed lot as well. Within a stratification period, however, no difference in % germination is apparent between seed pretreatments. The 24-hr. H₂O₂ treatment may be slightly more effective in germination than the 48-hr. running H₂O procedure at the lower stratification period (60-day).
- In general, the 120-day stratification period produced the most germination in younger seed lots. The 90-day stratification period, however, produced the most germination in 2 out of 4 of the older-aged seed lots.
- The two temperature regimes for germination, 20° night/22° C day (68°/72° F) vs. 16° night/18° C day (61°/64° F), were significantly different (p=0.04) but varied depending upon seed lot (p=0.02).
- Although age of seed is confounded by geographic origin, older seed lots had from 80-100% germination in some treatments.

A second study comparing germination of 35 seed lots of whitebark pine showed that % germination decreased with increasing latitude (p=0.005) and increased with decreasing longitude (p=0.02), thus of those sampled, germination was higher in southwestern populations. No relationship was found between % germination and age of population.

* age of seed lots is confounded by origin

CONCLUSIONS & DISCUSSION

- The 24-hr. H₂O₂ seed pretreatment utilized by Dorena Genetic Research Center is at least as effective for whitebark pine germination as the 48-hr. running H₂O control utilized by Coeur d'Alene Nursery.
- It was observed that the freshest seed lots produced a higher % of live seedlings than seed lots that had been stored for 3-7 years.
- 120 days appears to produce the most germination within seed lots and seed pretreatments compared to 60 or 90-days. It was also observed that a larger number of seed coats split without nicking, prior to the germination treatment, in the 120-day stratification treatment than in the other stratification treatments.
- Differences in germination temperature, as tested in this study, were a marginally significant factor in whitebark pine germination. It was noted, however, that mold occurrence was considerably lower in the cooler germination treatment.
- Dorena's operational default protocol for germinating whitebark pine will be: H₂O, soak, 120-day stratification, with a germination temperature of 16° night/18° C day (61°/64° F).

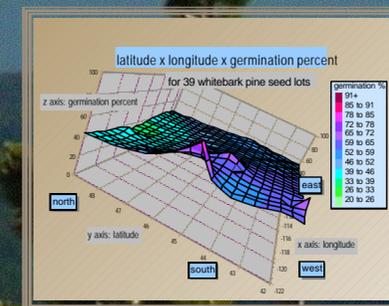
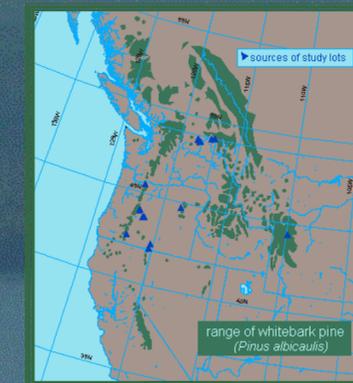
FUTURE RESEARCH

Continuation of this study will occur to evaluate culturing treatment. Height and diameter measurements will be made on all seedlings at the end of the growing season to evaluate the effect of climate-controlled vs. ambient growing conditions.

Seedlings from this study will be used in operation blister rust screening at Dorena.

LITERATURE CITED

Burr, KE, Eramian A, Eggleston K. 2001. Growing whitebark pine seedlings for restoration. In: Tomback DF, Arno SF, Keane RE, editors. Whitebark pine communities. Washington: Island Press. p 325-345.



1 month old whitebark pine seedling

photo credits: mikki coumas, bob danchok, jude danielson, ed jensen, kevin r. morris, lee riley, richard sniezko, diana tomback, and the whitebark pine ecosystem foundation

acknowledgements: We would like to acknowledge the support and contributions of the following: Dorena GRC Seed, Nursery, Rust, and Port-Orford-cedar crews for help in seed handling and germination monitoring; Whitebark pine cone collectors on the Colville, Colville, Deschutes, Fremont, Mt. Hood, and Umatilla National Forests; USFS Coeur d'Alene Nursery Seed Plant; as well as the continued support of the USFS Region 6 Genetics Program.