NURSERY REGIMES FOR DISEASE RESISTANCE SCREENING IN NORTH AMERICAN CONIFERS

I. BLISTER RUST (*Cronartium ribicola*) IN WESTERN WHITE PINE (*Pinus monticola*) AND SUGAR PINE (*P. lambertiana*)

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sugar pines in southern oregon

young sugar pine dying of blister rust

OBJECTIVES:

To provide optimum conditions for growing healthy seedlings for use in disease screening procedures. Following artificial disease inoculation, to provide conditions throughout the screening period to maintain vigor of trees with minimal interference with disease progression.

White Pine Blister Rust

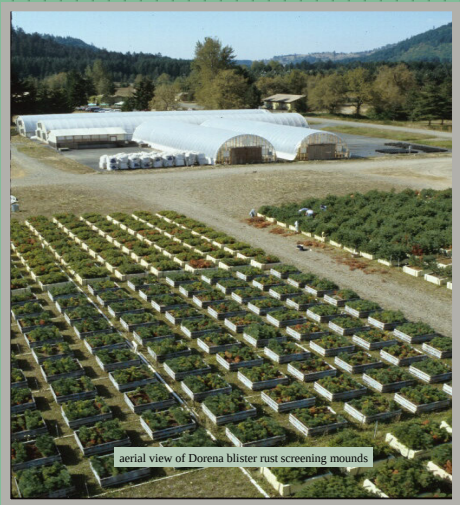
*In North America, blister rust is an exotic disease of 5-needled pines, primarily important in western white pine (*Pinus monticola*), eastern white pine (P. strobus), sugar pine (P. lambertiana), and whitebark pine (P. albicaulis). It was brought to the continent on nursery seedlings grown in Europe from North American seed in the early 20th century, to both the east and west coasts, and within 3-4 decades had decimated a large percent of native 5-needled pine populations. It continues to spread from the original infection centers in New York and British Columbia.*

The disease is caused by a fungus, Cronartium ribicola, *with a complex life cycle involving two very different plant hosts: the 5-needled pines and species of* Ribes *(currants and gooseberries). One assumption is that* Cronartium, Ribes, *and* P. cembrae *(Asian 5-needled stone pines) co-evolved in western Asia for millions of years in an area where* Ribes *was extant but not common. In the 16th and 17th centuries, both pines and* Ribes *for fruit production were extensively imported to Europe (many from North America), and a disease explosion occurred. The fungus was then transmitted to North America in 1909-10, where native species of both pines and currants were common and widespread in the same ranges.*

The disease cycle of blister rust is as follows: basidiospores produced on Ribes *leaves during late summer and fall are windborne in fog banks to pines, where they infect pine needles through the open stomata, forming lesions. By the following spring, fungal hyphae grow down the interior of the needle to the branch and into the cambium, eventually girdling and killing the branch or stem. A year or more later, the canker which has formed splits the bark and produces aeciospores which then are carried by wind to infect* Ribes *leaves in the spring. These spores go through multiplicative life stages on the currant leaves, and again produce basidiospores in the fall, which are carried to pines. The fungus is normally not fatal to the* Ribes, *which loses its leaves by winter.*

Natural resistance to infection and/or mortality exists in low to very low frequencies throughout populations of North American 5-needled pines. Since the 1940's, the strategy for returning these valuable pines to their ecosystems has been to make selections of phenotypically resistant individual trees from stands where blister rust is present, and either (1) propagate these directly by grafting of scion into seed orchards, or (2) collect seed from these individuals and sow them in randomized common garden test plots for screening, as is done at Dorena GRC.

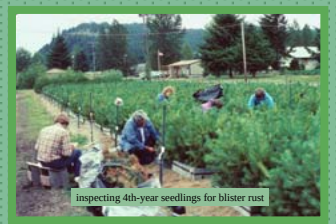
Progeny seedlings are cultured, artificially inoculated, and inspected for rust infection and resistance for 7 years. Survivors from the most broadly resistant families are then grafted into seed orchards for production of rust-resistant or rust-tolerant seed for reforestation.Controlled cross pollination and breeding is also conducted to elucidate the genetics of rust resistance.


aerial view of Dorena blister rust screening mounds

Screening Procedure

- Seedlots to be sown are determined in Dec/Jan, and put into stratification.
- Seeds are sown into wooden frames in March, following strict seedlot identification procedures at 6 randomized replications each of 10-seedling-row plots in 60 frames. Frames are covered with wire tops to prevent rodent and bird predation until germination.
- Seedlings are cultured intensively through the 1st season, and thinned to 1 per planting spot in spring.
- Procedures for strong growth and maintenance of all 60 seedlings per family where possible are maintained through the 2nd season, with attempts to avoid lammas growth (often unsuccessful, due to western Oregon's climate).
- In September, following the 2nd growing season, frames are moved by front end loader into an inoculation chamber set to 100% humidity and 65-68°F. They are acclimatized for 48 hrs, and then infected *Ribes* leaves are placed over them on screen tops. Spore drop is closely monitored with slides among the seedlings examined with a microscope at frequent intervals. When target spore load is reached, the leaves are removed, a frame at a time, and the seedlings remain in the chamber another 48 hrs to facilitate spore germination.
- Frames are returned to the outdoor growing mounds by front end loader..
- Seedlings are individually inspected and recorded for blister rust needle lesions in spring of their 3rd year. Trees are inspected again in fall for lesions and cankers.
- Seedlings are inspected and recorded annually for 4 more years for blister rust manifestations and resistance. Dead trees are cut out at each inspection and recorded; individual identity is chronicled and maintained.
- After year 7, inspections are complete, survivors of interest have scion taken for grafting into seed orchards, and the remaining saplings are rogued to 1-2 per family.
- Frames with remaining saplings are transferred to the "boneyard" as a clone bank and breeding arboretum.
- Saplings are maintained in their original medium set onto the ground for as long as possible (up to 20 yrs at present). Inspection for rust resistance and controlled cross pollinations continue as possible. Individual seedling identity is recorded and tracked throughout the screening and post-screening period. Saplings are maintained with irrigation, fertilization, and mechanical vegetation control.

infected white pine seedling with lesions


inspecting 4th-year seedlings for blister rust

Acknowledgements: All the technicians and professionals who have developed and implemented the programs at Dorena Genetic Resource Center since 1959.

SEEDLINGS FOR INOCULATION

Targets:
- 100 % healthy seedlings for inoculation
- Maintenance of trees throughout testing period
- Mobility for inoculation (seedlings moved to inoculation chamber after 2nd growing season)
- Little to no lammas growth prior to inoculation
- Maximum insect and disease prevention/control of non-target organisms
- Maintenance of identities throughout testing period

Constraints:
- Appropriate spacing for initial growth, inoculation, disease prevention, long-term culture through screening period
- Creation of artificial media which provides good drainage and will maintain structure for up to 7 years
- Limited dormancy induction options
- Timing of sowing, culture, and dormancy induction to meet inoculation windows
- Large amount of manual labor necessary (very little mechanization)
- Limited disease control option before and after inoculation
- Very low threshold for pest control
- Use of untreated river water for irrigation

historical culture: sawdust cold protection and high seedling density.

Culturing history

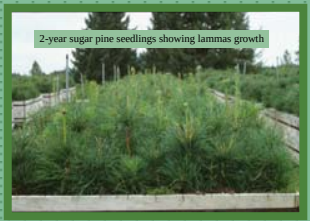
Seedling culture for rust resistance screening has undergone numerous changes over the course of the program. Changes in media, fertilizers, density, cold protection, etc. have all been made to improve the quality of stock for maximum screening success.

- *Media* - Peat:sand or peat:sandy loam mix (50:50).
  Problems: Required fumigation with methyl bromide. Did not provide adequate drainage.
  - Changed to peat:vermiculite mix (50:50).
  Problems: Break down of media within 3 years
- *Containers* - 48"x60"x10" plywood boxes on wooden pallets.
  Problems: Frames were too large. Pallets would deteriorate prior to movement of boxes following screening period.
- *Seed pre-treatment* - 24 hr soak in cold water. Stratify both species at 33-35°F (1-2 °C) for 90 days with water rinse once per month.
  Problems: Fungal infection. Uneven germination.
- *Spacing* - Hand sow at 3"x3" spacing (120 trees/frame).
  Problems: Tight spacing of trees contributed to the occurrence of the *Tuberculina* spp., a mold that restricts aeciospore production and potentially confounds screening results.
- *Fertilizer* - Granular mix of N-P-K with lime incorporated into bulk soil. "Rapid-gro" fertilizer in soluble form applied from 2nd year on (using tank sprayer).
  Problems: Uneven distribution of granular fertilizer into frames. Labor intensive addition of soluble fertilizer.
  - No granular fertilizer incorporated prior to sowing. Specialized soluble fertilizers applied through injection system during growing season.
  Problems: Soluble fertilizer applied outdoors resulted in uneven distribution (if any) into seedling frames – i.e. poor overall seedling growth and risk of nitrogen entering groundwater.
- *Cold Protection* - All seedling frames surrounded with sawdust.
  Problems: Increased potential for insect, disease, and weed problems. Seedling frames were less portable.

hand sowing of seeds in frames

sown frames covered with wire to prevent predation

germinated western white pine in frames


2-year sugar pine seedlings showing lammas growth

placing infected *Ribes* leaves over seedlings for rust inoculation

validation screening of resistant and non-resistant families

5th-year seedlings showing resistance and rust

the moveable forest: survivors transported to the "boneyard"

18-year-old white pine saplings in the "boneyard"

Current Culture

- *Media* – peat:vermiculite:fir bark:perlite:pumice.
  Advantage: Provides adequate drainage and structure throughout testing period. Does not require on site fumigation.
- *Containers* – 40"x48"x15" plywood boxes built on plastic shipping pallets.
  Advantage: Plastic pallets can be cleaned and reused in succeeding years.
- *Seed pretreatment* – 48 hr soak in 1% H_2O_2. Stratify SP at 33-35°F (1-2 °C) for 90 days and WWP at 50 °F (10 °C) for 30 days, then 33-35°F (1-2 °C) for 90 days. Rinse with water once per week, using 1% H_2O_2 when necessary.
  Advantage: Improved uniformness and speed of germination. Decreased fungal problems.
- *Spacing* – Hand sow at 4"x4" spacing (80 trees/frame).
  Advantage: Although new this year, the lower density should allow for increased air circulation and lower incidence of *Tuberculina*.
- *Fertilizer – Year 1* - Pre-sow fertilization with 6-7 month control release fertilizer. Addition of granular fertilizer (balanced with minors) as needed throughout growing season.
  - *Year 2 and beyond* – Application of control release fertilizer plus granular fertilizer (balanced with minors) in early March. Addition of granular fertilizer as needed throughout growing season.
  Advantage: Fertilizer is evenly distributed throughout the frames prior to sowing. Additional fertilizer in succeeding years is evenly applied over all frames and is available during entire period when needed. The regime is labor intensive in the spring, but does not require much additional labor through the growing season.
- *Pesticides* – Prevention and control of *Sirococcus*, *Phytophthora* spp., root weevils as needed.
- *Cold Protection* – Foam insulation placed around all frames containing SP throughout the winter season.
  Advantage: Decreased risk of insect, disease, and weed infestation. Foam can be easily removed each spring.