Influence of inoculum source and density on white pine blister rust infection and mortality of whitebark pine
2007 update on 2001 inoculation of Shoshone National Forest seedlings

Angelia Kegley, Richard A. Sniezko, and Robert Danchok

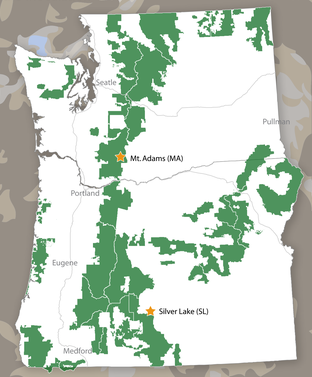
Whitebark pine seedling in restoration planting (photo courtesy of M. Jenkins)

Introduction

White pine blister rust is caused by the non-native invasive pathogen *Cronartium ribicola*. All nine North American five-needle pine species (*Pinus* subsection *Strobus*) are susceptible to this disease, with high mortality occurring in natural stands as well as plantings of *Pinus monticola* (western white pine), *P. lambertiana* (sugar pine), and *P. albicaulis* (whitebark pine). There is little published information about artificial screening of whitebark pine seedlings for blister rust resistance. In this trial, we examine whether the amount of basidiospores (inoculum density) or the geographic source of inoculum influence the resistance of whitebark pine. The purpose of this study was to help refine an inoculation protocol for operational screening of whitebark pine at Dorena Genetic Resource Center. This report updates the early results presented in 2003 and focuses on stem infection and rust mortality.

Onset of mortality

Figure 1. Locations of infected Ribes leaves used in the inoculation



Stem symptom on study seedling

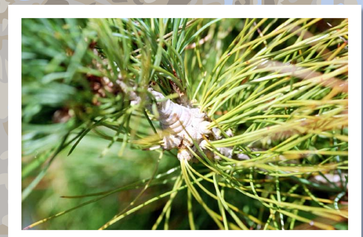
Table 1. Means by inoculum source and density

Inoculum Source[a]	Inoculum Density[b] Target	Actual (se)	# trees	% infected[c]	% SS	% SSAL	% RMORT	% survival
MA	1000	825 (165.2)	46	100.0	73.7	17.0	60.4	39.6
MA	2500	2500 (248.3)	49	96.0	85.6	18.7	69.3	24.4
MA	5000	5150 (585.2)	28	100.0	100.0	14.4	81.9	14.4
SL	1000	1000 (91.3)	47	100.0	95.8	19.8	76.2	23.8
SL	2500	2625 (342.5)	49	97.8	87.8	12.7	75.6	22.4
SL	5000	5400 (393.7)	37	100.0	95.0	11.8	83.8	16.3

[a] Where MA = Mt Adams, WA; SL = Silver Lake, OR

[b] Measured in basidiospores/cm^2 where 1000 represents a low, 2500 represents a medium, and 5000 represents a high density

[c] Percentage seedlings that developed needle lesions or stem symptoms



Stem symptom on study seedling. Note swelling at base of fascicle bundle

Materials and Methods

Plant Material

The whitebark pine seedlings were from a bulked seed collection (CDA # 7425) from the Shoshone National Forest southwest of Dubois, Wyoming (≈43°28'N 109°52'W, elevation ≈2987 m). Seed was sown in 1999 at the Forest Service's Coeur d'Alene nursery and transported to Dorena Genetic Resource Center (DGRC) in July 2001 just before the September 2001 inoculation.

Inoculation

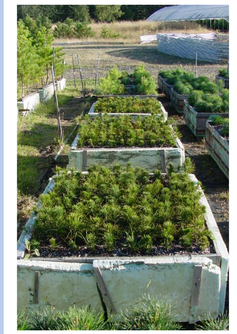
Treatments consisted of a factorial of 2 sources of inoculum (*Ribes* sp. leaves infected with *Cronartium ribicola* at the telial stage) (Figure 1) and 3 targeted inoculum densities (Table 1). Seedlings were divided into six groups with approximately 48 seedlings per group; each group was allocated to one of the six treatment combinations (Table 1). The inoculation treatments were randomly assigned in the chamber with no replication. Inoculation followed standard DGRC procedure (Danchok *et al.* 2004). The seedlings were transplanted into three standard DGRC boxes approximately 3 weeks after inoculation. Each treatment was randomly assigned to five rows in a completely randomized design.

Assessments

Seedlings were assessed in June 2002 (≈9 months after inoculation) for height (cm) and number of needle lesions. Number of needle lesions, number of stem infections, and survival were assessed in March 2003 (≈18 months after inoculation) and July 2003 (≈22 months after inoculation). Survival and presence of stem symptoms were assessed approximately 30, 34, 46, and 52 months after inoculation. Only percentage seedlings with stem symptoms and mortality are reported here.

Analysis

Exploratory analyses of variance using SAS Proc GLM (SAS 1999) for the percentage seedlings with stem symptoms and percentage seedlings surviving were performed using plot means.



Transplanted whitebark pine seedlings

Results

Stem symptoms

Only relatively small differences existed between the 2 widely separate geographic sources of inoculum for percentage of trees with stem symptoms (% SS); the Mt. Adams (MA) source averaged 86.4% SS, and the Silver Lake (SL) source averaged 92.9% SS.

There were only slight differences in means for the three inoculum densities for % SS for the SL source (87.8 to 95.8%) but larger differences for the MA source (73.7 to 100%) (Table 1).

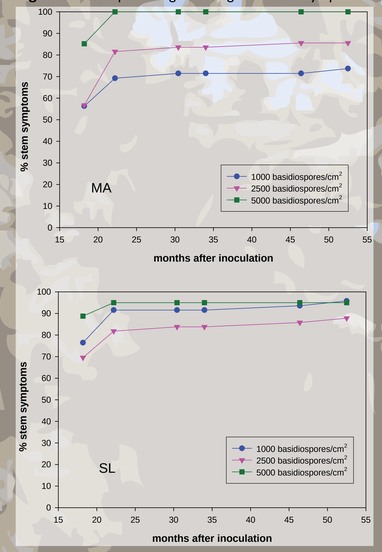
In general most of the seedlings developed stem symptoms by the 22-month assessment (Figure 2).

Mortality

There was very little difference in mortality between the two inoculum sources; the MA source averaged 70.5% rust mortality (% RMORT), while the SL source averaged 78.5% at 52 months after inoculation.

As with % SS, there were slight differences in means for the three inoculum densities in % RMORT for the SL source (75.6 to 83.8%) and larger differences for the MA source (60.4 to 81.9%) (Table 1, Figure 3).

Figure 2. Mean percentage seedlings with stem symptoms



Summary

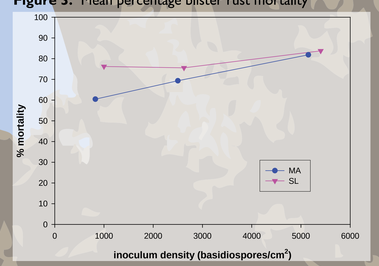
In resistance testing, there is a balance between minimizing escapes (seedlings that do not get infected due to factors in the inoculation process) and overwhelming seedlings with too much inoculum. Based on 40 years of experience with inoculating other white pine species and the results here, we will use ≈3000 spores/cm^2 for operational testing of resistance. The source of spores appears to have little influence on the final outcome.

Other trials currently underway and further analysis of this trial (including its limitations) will allow us to evaluate further the optimum density of spores and whether geographic source of spores has any major influence on results.

The level and types of resistance in the Shoshone NF seedlot used here are still under investigation. A recent and larger study examining the effects of inoculum source and inoculum density has been undertaken. In addition, the survivors of this prototype trial were reinoculated in 2005 and will be assessed for development of disease symptoms and mortality.

Application of infected Ribes leaves for inoculation

Figure 3. Mean percentage blister rust mortality



Survival of inoculated seedlings in 2006

Whitebark pine restoration planting (photo courtesy of E. Jungck)

USDA Forest Service
Dorena Genetic Resource Center
Cottage Grove, OR 97424
http://www.fs.fed.us/r6/dorena/

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References

Danchok, R., Sharpe, J., Bates, K., Fitzgerald, K., Kegley, A., Long, S., Sniezko, R., Danielson, J., and Spence, R. 2004. Operational manual for white pine blister rust inoculation at Dorena Genetic Resource Center. 30 p.

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