Influence of Inoculum Source and Density on White Pine Blister Rust Infection and Mortality of Whitebark Pine: 2007 Update on 2001 Inoculations of Shoshone National Forest Seedlings

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Abstract

Artificial inoculation trials to evaluate genetic resistance of whitebark pine have only recently started. Inherent in such trials are questions of how results vary with inoculum source or inoculum density. Seedlings from a bulked collection of cones from trees on the Shoshone National Forest were inoculated in 2001. Treatments consisted of a factorial of two geographic sources of inoculum and three levels (densities) of inoculum. Early results showed high infection levels (needle spots and/or cankers) across all treatments within a year of inoculation. Through March 2006, the percentage of trees with stem symptoms for the six treatment combinations varied from 73.7 to 100 percent, while rust mortality varied from 60.4 to 83.8 percent. Infection and mortality levels were similar for the mid- and high inoculum density treatments. The surviving trees were re-inoculated in September 2005 and will be followed further to examine the level of resistance in this Shoshone population.

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 western white pine (*Pinus monticola*) and sugar pine (*P. lambertiana*). There is little published information about artificial screening of whitebark pine (*P. albicaulis*) 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 2004 and focuses on stem infection and rust mortality.

Materials and Methods

Plant Material

Whitebark pine (WBP) seed from a bulked cone collection (CDA # 7425) from the Shoshone National Forest southwest of Dubois, Wyoming (~43°30'N 109°50'W, elevation ~2987 m) was sown in Ray Leach SC-10-Super Cells (16.4 cm^3) in April 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) 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 inoculation chamber with no replication and were separated to minimize cross-contamination. Inoculation followed standard DGRC procedure (Danchok and others 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, with 8-10 seedlings per treatment row.

Table 1. Treatments of whitebark pine inoculated in September 2001.

Ribes Source[a]	Inoculum Density (basidiospores/cm^2)			Spore Germ[b] (%)	Inoc. Time (h)[c]
	Level	Target	Actual (se)		
MA	Low	1000	825 (165.2)	95	15.5
MA	Medium	2500	2500 (248.3)	87	26.5
MA	High	5000	5150 (585.2)	99	40.5
SL	Low	1000	1000 (91.3)	99	11.0
SL	Medium	2500	2625 (342.5)	100	47.0
SL	High	5000	5400 (393.7)	87	37.3

[a] Ribes sp. leaves collected from Silver Lake, Oregon (SL) and Mt. Adams, Washington (MA)
[b] Basidiospore germination
[c] Time (h) to reach target inoculum density

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, percentage survival with stem symptoms, and percentage rust mortality are reported here.

Analysis

Exploratory analyses of variance using SAS Proc GLM (SAS Inc. 2006) for the percentage seedlings with stem symptoms and percentage seedlings surviving were performed using untransformed plot means. Inoculum source information was lost for 3 of the high density plots; those plots were excluded from this summary.

Results

Stem Symptoms

At 52 months after inoculation percentage seedlings with stem symptoms (% SS) ranged from 73.7 to 100% for the Mt. Adams (MA) inoculum source treatments, and 87.8 to 95.8% for the Silver Lake (SL) treatments (Table 2). For the MA treatments there was an increase in % SS as inoculum density increased (Table 2, Figure 1). For the SL treatments both the high and low density treatments had slightly higher % SS than the medium density treatment (Figure 1). The overall mean for SL was slightly greater than for MA (92.9 vs. 86.4% SS, respectively). In general most of the seedlings had developed stem symptoms by the 22-month assessment, regardless of treatment (Figure 1).

Table 2. Means by inoculum source and density

Inoculum Source[a]	Inoculum Density	# seedlings[b]	% infected[c]	% SS	% SSAL	% RMORT
MA	Low	46	100.0	73.7	17.0	60.4
MA	Medium	49	96.0	85.6	18.7	69.3
MA	High	28	100.0	100.0	14.4	81.9
SL	Low	47	100.0	95.8	19.8	76.2
SL	Medium	49	97.8	87.8	12.7	75.6
SL	High	37	100.0	95.0	11.8	83.8

[a] Where MA = Mt Adams, WA; SL = Silver Lake, OR
[b] Inoculum source information was lost for 3 plots inoculated at the high density
[c] Percentage seedlings that developed needle lesions or stem symptoms (SS)

Four years after inoculation differences between inoculum sources were not significant for % SS; there were significant differences between inoculum sources at the 18-month assessment only. In contrast the main effect of inoculum density was significant until the 52-month assessment (F=2.28, p=0.0670).

There was a significant interaction between inoculum source and inoculum density for the last two assessments (F=3.52, p=0.0481; F=3.67, p=0.0428). This is likely driven by the high % SS in the SL low density (1000 basidiospores/cm^2) treatment and differences in scale between inoculum sources.

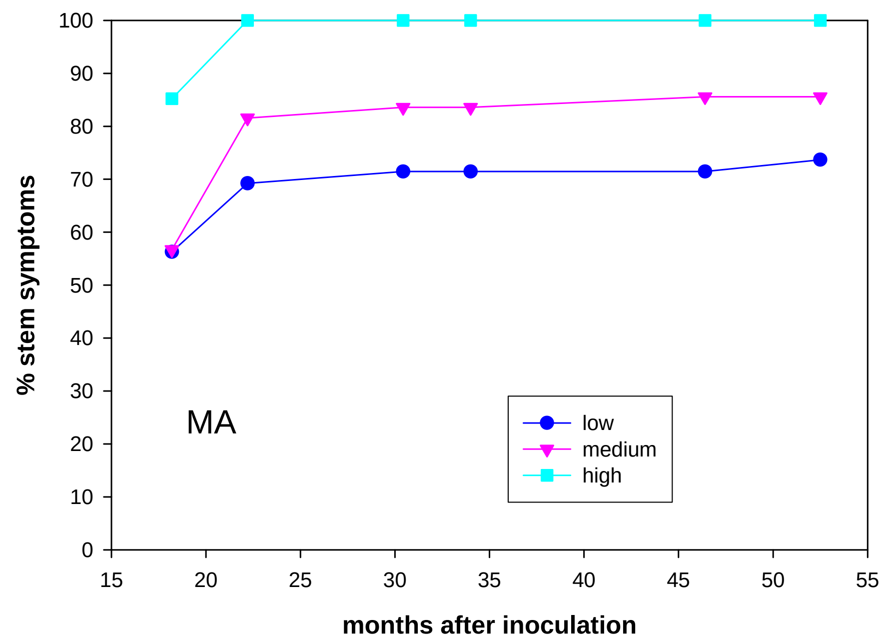
The percentage seedlings surviving with stem symptoms at 52 months after inoculation (% SSAL) ranged from 11.8 to 19.8% among the treatments. For the MA treatments % SSAL was similar for the Low and Medium density treatments and slightly lower for the High density treatment (Table 2). However, for the SL treatments, % SSAL was higher for the Low density treatments and lower for the Medium and High density treatments (Table 2).

Mortality

Rust mortality (% RMORT) means ranged from 60.4 to 81.9% for the MA treatments and from 75.6 to 83.8% for the SL treatments at 52 months after inoculation (Table 2, Figure 2). For the MA treatments there was an increase in % RMORT as inoculum density increased (Table 2, Figure 2). % RMORT was relatively similar across inoculum densities for the SL treatments 46 and 52 months after inoculation (Table 2, Figure 2). The SL treatments tended to have slightly higher % RMORT (78.5 vs. 70.5%, respectively), but this difference was not statistically significant. There were significant differences among inoculum sources in % RMORT at the 30-month assessment only (F=4.57, p=0.0445).

The high density treatment had the highest % RMORT, regardless of inoculum source, at all assessments (Table 2, Figure 2). % RMORT was similar between the Low and Medium density treatments within a given inoculum source. Although % RMORT is higher in the high density treatments, regardless of inoculum source, this difference was not statistically significant. There were significant differences among inoculum densities at the 30 and 34 month assessments only (F=5.42, p=0.0127; F=6.37, p=0.0069).

Figure 1. Treatment mean percentage seedlings with stem symptoms (% SS) by inoculum source



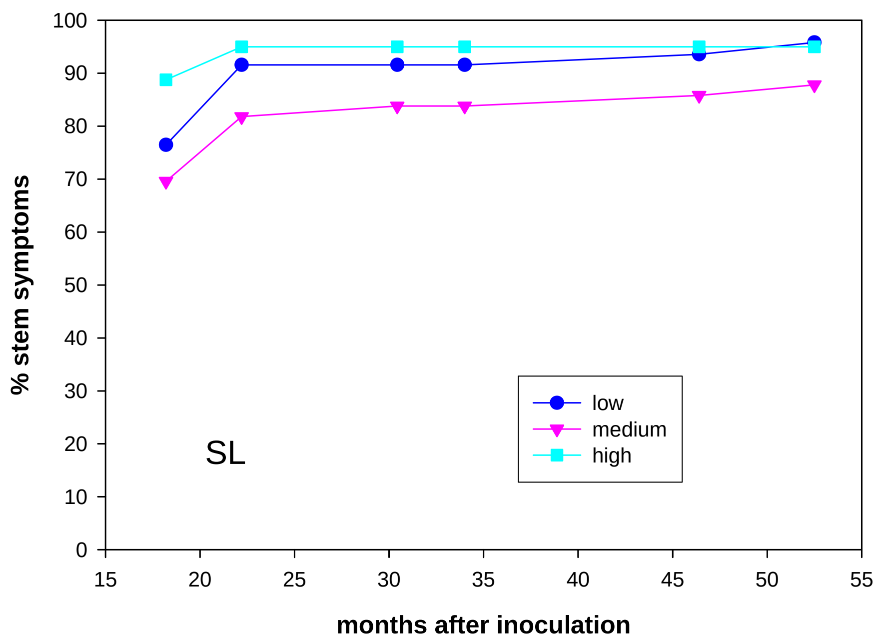
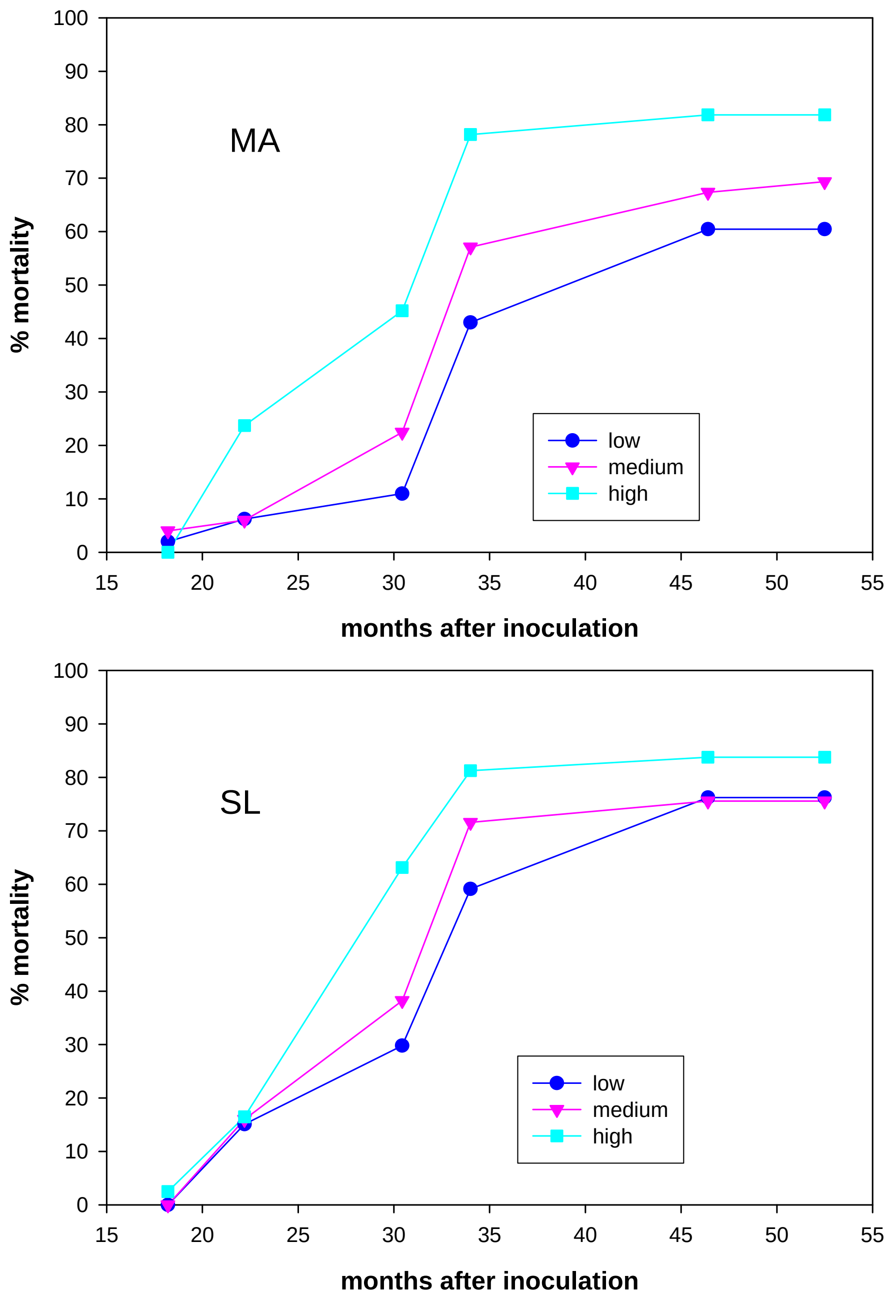


Figure 2. Treatment mean percentage rust mortality by inoculum source



Discussion and Summary

These results indicate that although there may be small but significant initial differences between geographic sources of rust or among inoculum densities, these effects are transitory for the traits % SS, % SSAL, and % RMORT. These results report only the percentage seedlings with stem symptoms or dead of blister rust. It should be noted that there were typically two to three times as many stem symptoms per infected tree in the high density treatments relative to the low and medium density treatments, regardless of inoculum source (Kegley and others 2004). Although the final ratings may be similar, there may be differences in ontogeny of symptom development among inoculum sources (Kegley and others 2004). The effect of inoculum density was more persistent for % SS, only losing significance at the 52-month assessment.

Mortality only reached ~ 85% for the high density treatments through 52 months after inoculation; this is slightly unexpected given the high infection achieved in the test (>96%). Typically, open-pollinated progeny of phenotypic selections of western white pine and sugar pine exhibit >90% mortality in operational screening at DGRC (unpublished data). Thus, there may be at least a low level of resistance present in the Shoshone WBP population. In a subsequent DGRC trial, the Shoshone bulked seedlot showed relatively low levels of resistance and low survival through 2 years after inoculation (10 and 11.8% survival for DGRC-grown and Coeur d'Alene-grown seedlings, respectively) (Sniezko and others, this proceedings). However, the Shoshone bulked seedlot was ranked 59 of 108 seedlots for blister rust resistance in a test of WBP from the Inland Northwest (Mahalovich and others 2006).

Based on the data from this study, an inoculum density of 3000 basidiospores/cm^2 will ensure high levels of infection needed to test for resistance, while minimizing potential escapes and the possibility of overwhelming resistances that are manifested at moderate inoculum densities. Geographic source of inoculum appears to have little effect on the final outcome, indicated by rust mortality. This was a relatively small study, with a maximum of 48 seedlings per treatment combination, so the conclusions reached here are merely broad guidelines. Both geographic sources of rust were in the inoculation chamber at the same time, so there was the possibility of contamination among the treatments. However, the results presented here are similar to early results reported by Sniezko and others (this proceedings) for two separate trials; survival and % SS were similar for seedling families exposed to two different sources of inoculum. A trial examining the effect of inoculum density on individual seedling families has also been undertaken (unpublished data). In addition, the survivors of this prototype trial were reinoculated in 2005 and will be assessed for development of disease symptoms and mortality. This will help ascertain whether there were some escapes in the first inoculation.

Acknowledgments
Melissa Jenkins from the Caribou/Targhee NF provided the whitebark pine seedlings, and Ellen Jungck of the Shoshone NF provided background information on the seed collection. The Coeur d'Alene Nursery grew the seedlings for the first two years. Dorena GRC technicians and staff inoculated the seedlings with blister rust, provided nursery culture after inoculation, and assessed the seedlings. The USDA Forest Service Region 6 Genetics and Forest Health programs provided funds to carry out the study. Ryan Berdeen designed the poster (available for download at http://www.fs.fed.us/r6/dorena/documents/publications/pub131.pdf).

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