Variation in Blister Rust Resistance and Early Height Growth in Three Populations of Southwestern White Pine (*Pinus strobiformis*) and Implications for Management and Conservation

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Abstract—Southwestern white pine (SWWP, *Pinus strobiformis*), a long-lived conifer in Mexico and the southwestern United States, is very susceptible to the nonnative pathogen *Cronartium ribicola*, the cause of white pine blister rust. Due to the recent introduction of *C. ribicola* to the Southwest, the future viability of many populations of SWWP is in jeopardy. Fortunately, some early studies showed that there is at least a low frequency of genetic resistance in SWWP. In partnership with the U.S. Department of Agriculture, Forest Service (USDA FS), Southwestern Region Forest Health Protection and colleagues at Northern Arizona University, several trials to evaluate genetic resistance to the rust are now underway at the USDA FS Dorena Genetic Resource Center (Cottage Grove, Oregon, USA). We summarize here early results from a seedling inoculation trial involving 40 half-sib families from three populations in New Mexico. Sources of the three populations were the Lincoln, Cibola, and Santa Fe National Forests. Seventeen of the 20 selections from the Lincoln National Forest were from putatively canker-free trees present within a heavily infested stand; the three others were cankered trees and serve as susceptible controls. There is currently a low incidence of rust infection on the Cibola and Santa Fe National Forests, and the 20 parents selected there were considered random selections. Data collected in this experiment show differences between the tested SWWP populations in early height growth as well as resistance to *C. ribicola*. The Lincoln National Forest population was taller and more resistant to *C. ribicola* than the randomly collected populations from the Cibola and Santa Fe National Forests.

INTRODUCTION

Populations of southwestern white pine (SWWP, *Pinus stobiformis*), a long-lived conifer native to the American Southwest and Mexico, are increasingly threatened by white pine blister rust (WPBR) disease, caused by the nonnative fungal pathogen *Cronartium ribicola* (fig. 1). This disease was first reported causing damage to SWWP in the Sacramento Mountains of southern New Mexico in 1990 (Hawksworth 1990). Since then, the fungus has spread throughout central and southern New Mexico and the White Mountains of Arizona (Conklin et al. 2009; Fairweather and Geils 2011). On the Lincoln National Forest in southern New Mexico, incidence of infection has exceeded 80 percent in some areas (Conklin 1994, 2004), with many infected trees in advanced stages of decline. Some large mature trees had hundreds of cankers (fig. 2) and topkill (Conklin 2004).

Both major gene resistance and partial resistance have been previously documented in this species (Hoff et al. 1980; Kinloch and Dupper 2002; Sniezko et al. 2008), but relatively few families have been evaluated for partial resistance. In this common garden study

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Figure 1—Southwestern white pine (a) sapling with several branch cankers, (b) bole canker with aecia, and (c) large tree with many cankers and top kill from rust (photo credits: Mary Lou Fairweather (a & b); Betsy Goodrich (c)).

we examine variation in early height growth and resistance to WPBR among half-sib seedling families of SWWP originating from three populations in New Mexico: Cibola (CNF), Santa Fe (SFNF), and Lincoln (LNF) National Forests. The study is ongoing, and we report here only the early trends among the three forest populations in seedling growth, effectiveness of the artificial inoculation, and some elements of early resistance.

METHODS

Seed from 40 families of SWWP from the CNF, SFNF, and LNF were sown in March 2009 at the U.S. Department of Agriculture, Forest Service (USDA FS) Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA). The seedlots were individual tree collections from 10 parent trees each from the CNF and the SFNF and from 20 parent trees on the LNF. Phenotypically canker-free SWWP surrounded by heavily infected individuals had been previously documented in the Bradford Canyon area of the LNF (Conklin et al. 2009). The parent trees from the LNF included 17 putatively resistant trees that did not exhibit observable cankering and 3 trees that had moderate-to-heavy cankering (to serve here as susceptible controls). The parent trees from the CNF and SFNF populations were in areas with little or no rust at the time of selection and represent random selections from those populations.

Seedlings were grown for two growing years, 1 year in supercell Cone-tainers™ (Ray Leach, Canby, Oregon) (164 cm3) in family blocks in the greenhouse,

Figure 2— "Discovery Tree"—the first infected *Pinus strobiformis* tree found in the Southwest, as it appeared in 1990. Branch 'flagging' from hundreds of blister rust cankers is from 5–6 year-old infections. The tree is located 2 miles NE of Cloudcroft, on the Lincoln National Forest (photo: Dave Conklin).

followed by transplanting to 0.9 m × 1.2 m × 0.3 m boxes outside for the second growing season (fig. 3). Twelve to 60 seedlings were available for each family (mean = 47), and seedlings were transplanted into family row plots in a randomized complete block design with 6 blocks and up to 10 seedlings per family per block. Seedlings were inoculated in September 2010 with basidiospores of *C. ribicola* (fig. 4) (see Kegley and Sniezko 2004 and Sniezko et al. 2008, 2011 for general protocols used in inoculations at DGRC). Mean inoculum density was 4,527 spores/cm^2; basidiospore germination was 98.7 percent. Both primary and secondary needles were present at the time of inoculation.

Seedlings were assessed in spring 2011 for second-year height (HT2), number of needle spots on secondary needles and primary needles, and number of stem symptoms (cankers or bark reactions). Additionally, the percentage of secondary needle necrosis caused by needle blight fungi and environmental factors was recorded for each seedling. Subsequent annual assessments were done in late fall and early winter of 2011, 2012, 2013, and 2014; presence and number of stem symptoms by type (normal canker or bark reaction), severity of stem symptoms, and mortality were recorded. Third-year height (HT3) was recorded in fall 2011; this was the new growth in the year following inoculation with rust, including any late-season lammas growth. The severity score quantified the cumulative impact of all the stem symptoms for a seedling at each assessment. It ranged from 0 to 9 (where 0 = no stem symptom; 1 to 4 = very small or nongirdling infection(s); 5 = at least one stem symptom encircling the diameter of the seedling, but little vertical extension; 6 to 8 = extensive cankering encompassing much of the seedling radially and vertically; 9 = seedling dead from rust). Here, we report on some assessments through winter 2011, using the four groups (populations) of families: (1) CNF, (2) SFNF, (3) LNF-R (from canker-free parents), and (4) LNF-S (three susceptible controls).

Statistical analyses were conducted by using SAS® 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The analyses of variance (ANOVAs) for heights were based on family-plot means using PROC MIXED. Forests and families nested within forests were considered fixed effects; block effects were considered random. For HT3, the severity of rust was added to the model as an explanatory variable. For analyses on binomial responses such as mortality, a logistic regression model with logit link was fit using PROC GLIMMIX, with forests treated as fixed effects and random block effects.

RESULTS

There were statistically significant differences among the four populations and among families within populations for both second-year height (pre-inoculation, $P < 0.0001$ for both) and third-year height (1 year post-inoculation, $P < 0.0001$ for both). Seedlings from the LNF-R were on average taller (20.1 cm for the 17 resistant parents) than those from CNF (19.2 cm) or SFNF (18.2 cm) or those from the three susceptible parents on the LNF (18.5 cm) for HT2. Some of the height differences between families within a population were also visibly striking (fig. 5). In this trial,

Figure 3—Southwestern white pine (*Pinus strobiformis*) trial shortly after artificial inoculation with *Cronartium ribicola*.

Figure 4—Southwestern white pine (*Pinus strobiformis*) in Dorena Genetic Resource Center 'fog' chamber just prior to artificial inoculation with *Cronartium ribicola*.

Figure 5—Large within-population variation in height growth after second growing season for two families from Cibola National Forest. Family '49' is taller (row plot on left, mean 2nd year height = 23.5cm) and the much shorter Family '45' (row plot on right, mean height = 17.7cm).

there was a significant negative correlation between third-year height and the severity of rust infection at the time of inspection ($P = 0.002$). Seedlings with a one-unit higher canker severity score were on average 0.9 cm shorter (95-percent confidence interval from 0.3 to 1.4 cm shorter).

The inoculation of the seedlings with *C. ribicola* was very effective in this experiment, with 97.3 and 99.6 percent of seedlings exhibiting characteristic needle spots on the primary and secondary needles, respectively (fig. 6). The mean number of needle spots per seedling on secondary needles was 40.9 at 9 months post-inoculation (mpi).

Stem symptoms, cankers, and bark reactions appeared relatively quickly; 56.9 percent of seedlings had stem symptoms 9 mpi and 87.0 percent had stem symptoms at 15 mpi (fig. 7). At second assessment (~15 mpi), the seedling progenies from the LNF-R averaged 75.7 percent with stem infections versus 94.1, 98.0, and 90.7 percent with stem infections for those from the CNF, SFNF, and LNF-S, respectively; the mean number of stem infections per family was 11.2 (ranging from 2.9 to 20.7 for the 40 half-sib families). Mean severity of rust ~15 mpi varied from 3.9 for LNF-R to >6.0 for the other three populations in this study.

The percent secondary needle necrosis present in early spring 2001 in this study varied significantly for the four groups of seedling families studied ($P < 0.0001$), with 54, 67, 59, and 68 percent of seedlings affected on the LNF-R, LNF-S, CNF, and SFNF, respectively. The mean percentage of secondary tissue affected overall was 8.2 percent. Only 10.4 percent of seedlings had more than 20 percent of secondary tissue affected.

Figure 6—Southwestern white pine (*Pinus strobiformis*) seedling from Santa Fe National Forest (Sow #59) with hundreds of needle spots after blister rust inoculation; this family had 41 needle spots per seedling and 94.4 percent of seedlings with stem symptoms (and 13 stem symptoms per seedling) at 9 and 15 months post-inoculation, respectively.

After accounting for forest differences, there was no evidence of a relationship between either the probability of a seedling exhibiting needle necrosis or the percentage of secondary tissue affected and the percentage of seedlings cankered ($P = 0.54$ and $P = 0.90$, respectively).

Eight percent (150 of the 1,872) of the trees in the trial were killed by *C. ribicola* over the 15-mpi monitoring period reported here. This mortality varied significantly by population ($P < 0.0001$), from 2.5 percent for LNF-R, to 7.5 percent for LNR-S and 12.5 percent for both CNF and SFNF. One family from CNF had 36 percent rust-related mortality at this early stage.

DISCUSSION

Differences in early height growth were found between the populations and may suggest a need for establishing breeding zones within this species. Other trials are now underway to examine in more detail the differences in some adaptive traits in this species (Goodrich et al., this proceedings). The rapid development of cankers during the first growing season after inoculation affected normal season growth, the lammas growth at the end of the growing season, or both; seedlings with the highest severity ratings for rust showed the most impact to growth in the 15 months following inoculation.

Figure 7—Southwestern white pine seedling from Cibola National Forest family (Sow #50) with numerous stem cankers 14 months after inoculation; this family had 58 needle spots per seedling (9 months post-inoculation) and 94.8 percent of trees with stem symptoms and 17 stem infections per seedling, at ~15 months post-inoculation.

The overall frequency of canker-free seedlings was low (<6 percent) at 15 mpi in the seedling families from the parental selections from the SFNF and CNF, populations which originated from stands with little or no blister rust present. A low frequency of canker-free trees was also found in several earlier seedling inoculation studies of SWWP resistance to WPBR in the United States (Hoff 1980) and Germany (Stephan 1986). These data and the high infection (>80 percent) in some natural stands (Conklin 2004) confirm the high susceptibility of SWWP and the relatively low incidence of genetic resistance to WPBR in this species. Seedling families from the canker-free parent trees on the LNF had the lowest levels of early stem infection (mean = 75.7 percent) following artificial inoculation, but several families from this population had more than 90 percent of seedlings with stem symptoms. The parent trees from LNF are in an area of very high incidence and severity of rust infections, and 17 of the 20 selections tested were canker-free at the time of seed collection. Efficacy of selection was expected to be higher in this population than that from the other two forests given the presence of *C. ribicola* within the LNF for decades before selection, and this holds true for the early component of resistance reported here. A smaller, earlier trial of progeny from other parent trees from LNF also indicated a relatively high frequency of resistance, including a relatively low percentage of trees with stem symptoms (42.5 percent) 39 mpi (Sniezko et al. 2008). The lower level of stem symptoms in the earlier trial may be due to the different families utilized, a higher effective inoculation in the trial reported in this paper, or a combination thereof. In previous seedling testing at the USDA FS Institute of Forest Genetics (Placerville, California), progeny of the 17 canker-free LNF parents showed no evidence of 1:1 segregation for major gene resistance (D.A. Conklin, personal communication, 2008). The primary needles (which are more susceptible than secondary needles) present on the seedlings in this test may have led to somewhat higher stem infection than if only secondary needles had been present. Regardless, selections from the canker-free LNF parents were more resistant to WPBR in this trial than those from the other two geographic areas. Field trials to confirm the actual level and durability of resistance are needed before extrapolating any seedling assay such as this one to long-term field results.

This trial is still ongoing, but large differences among families and populations in resistance to WPBR are apparent. Analyses are currently underway, and in later summaries we will discuss the family and seed source variation in additional rust-resistance traits over the 5-year post-inoculation assessment period. In this study we observed necrosis on the secondary needles for each of the seedling families. The data indicated that although a moderate frequency of needle necrosis caused by an unknown incitant was present in each of the seedling families, it affected only a small percentage of the secondary needle tissue on most seedlings. The incitant probably had very little impact on cankering incidence, as we did not observe any association between percent necrosis and percent cankered. An additional 158 families from throughout the U.S. portion of the range of SWWP were sown in 2014 for blister rust inoculation in 2015, and this will provide a broader estimate of the frequency and distribution of resistance to *C. ribicola* in this species.

Field trials to validate resistance observed in seedling inoculation trials and to examine its durability are planned. Seed collections for gene conservation have begun, and studies of genetic variation in adaptive traits are underway (Goodrich et al., this proceedings). Other management options for mitigating the effect of WPBR in natural stands have been discussed elsewhere (Conklin et al. 2009). Some of the high-hazard sites in the Southwest may necessitate planting of resistant seedlings, but much can also be done by favoring white pine in silvicultural treatments. Although thinning of stands can decrease the likelihood of stand-replacing fire and thereby conserve possible resistant/tolerant white pines, it also increases the understory sunlight and in some areas, especially when combined with underburning, may lead to an increase in populations of *Ribes* species, the alternate host for *C. ribicola*. Fortunately, there are some forested areas in the Southwest with little to no presence of susceptible *Ribes* species (M.L. Fairweather email to Sniezko, Feb. 12, 2015; Geils et al. 1999; Looney 2012; van Arsdel et al. 1998).

Southwestern white pine is very susceptible to white pine blister rust, and the frequency of naturally occurring resistance is low. Overall forest management goals, as well as an understanding of the natural resistance present in SWWP, will have to be considered if the region's white pine resources in many areas are to remain viable. Given the relatively recent introduction of WPBR to the Southwest, it is imperative that we identify and quantify resistance in this species, including the frequency of occurrence over the landscape. Based on permanent plot monitoring, it is clear that retaining SWWP on high-hazard sites will be difficult without resistant planting stock. The first rust-resistant parents have now been identified, and seed can be collected from them to use for reforestation and restoration. These selections should also be grafted into orchards or clone banks to ensure retention of these valuable resistant parents.

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