

Pinus strobiformis Gene Conservation and Genecology

B.A. Goodrich¹, K.M. Waring^{1, *}, and L. Flores-Renteria²

¹ Northern Arizona University, School of Forestry, Flagstaff, Arizona, USA

² San Diego State University, Department of Biology, San Diego, California, USA

* kristen.waring@nau.edu

INTRODUCTION

Southwestern white pine (*Pinus strobiformis*, syn. *Pinus flexilis* var. *reflexa*) is a high elevation five-needle pine. *Pinus strobiformis* occurs in mixed species stands across a range of elevations and forest types in western Texas, southwestern Colorado, and throughout New Mexico and Arizona (USA), and continues along the mountains south to central Mexico (Alexander et al. 1984; Looney and Waring 2013; Moreno-Letelier and Piñero 2011; Steinhoff and Andresen 1971) (fig. 1). This species, like all species of North American five-needle pines, is susceptible to infection and mortality from the nonnative, invasive forest pathogen (*Cronartium ribicola*) that causes the disease white pine blister rust (WPBR) (Kinloch and Dupper 2002; Sniezko et al. 2011). White pine blister rust was first reported in natural stands of *P. strobiformis* in 1990 (Hawksworth 1990), and is spreading through the range in New Mexico and Arizona (Conklin 2004; Conklin et al. 2009; Fairweather and Geils 2011; Looney and Waring 2012). Across the area where WPBR was first discovered, about 40,500 ha have high WPBR incidence and trees with high severity infections including frequent “topkill” (a condition in which needles, branches, and stem tissue above the canker location are killed) (Conklin et al. 2009).

According to available species climate profile predictions, the suitable range of *P. strobiformis* is expected

to decrease under most future climate change scenarios (Crookston and Rehfeldt 2008). Predictions of future WPBR distributions are not as well understood because of difficulty in modeling pathogen/host tree interactions (Sturrock et al. 2011), but high hazard areas exist in the Southwest (Conklin 2004; Geils et al. 1999).

The impacts of WPBR on five-needle pine species in northern Rocky Mountain and Pacific Northwest ecosystems illustrate the disastrous effects of the disease on susceptible hosts on high hazard sites (Fins et al. 2002; Tomback and Achuff 2010). Cascading negative effects of forest recovery and ecosystem services in these areas should be considered a preview of what could occur in some southwestern ecosystems with no intervention (Schoettle and Sniezko 2007; Tomback and Achuff 2010). Negative effects may be exacerbated both through reductions of *P. strobiformis* genetic diversity with declining suitable habitat and through local population extirpations from recent large, high-severity fires across the Southwest (fig. 1).

Intervention in this case refers to the use of proactive restoration, where silvicultural practices can be used to promote natural (stimulated) or artificial (planted) regeneration with some resistance to WPBR (Keane and Schoettle 2011; Schoettle and Sniezko 2007). Our program to sustain *P. strobiformis* in southwestern

In: Schoettle, Anna W.; Sniezko, Richard A.; Kliejunas, John T., eds. 2018. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobosphere; 2014 June 15–20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 245 p.

Papers published in these proceedings were submitted by authors in electronic media. Editing was done for readability and to ensure consistent format and style. Authors are responsible for content and accuracy of their individual papers and the quality of illustrative materials. Opinions expressed may not necessarily reflect the position of the U.S. Department of Agriculture.

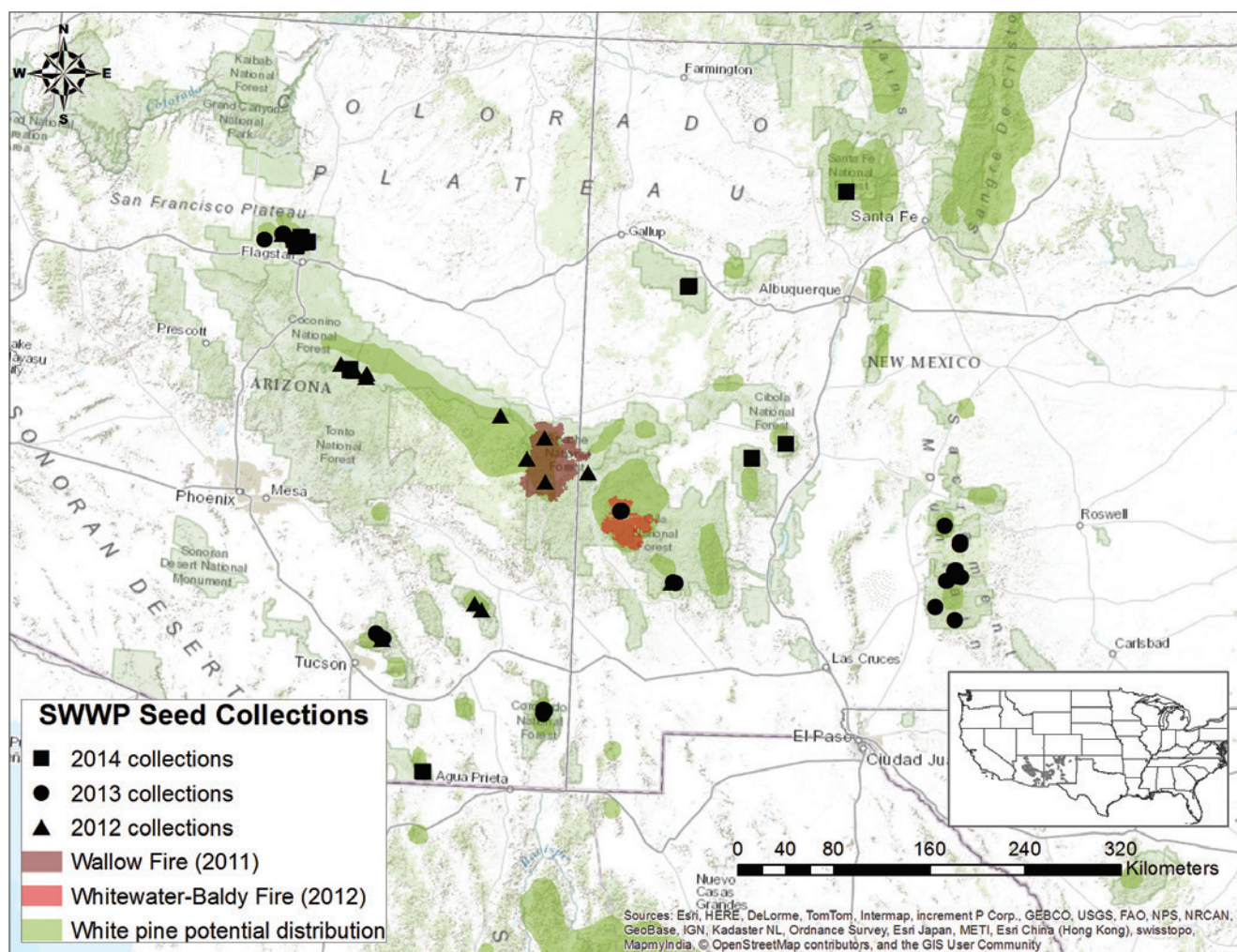


Figure 1—*Pinus strobiformis* (southwestern white pine, SWWP) gene conservation seed collection sites across USDA FS Region 3; collections from 2012–2014. Recent large fires in collection areas / *P. strobiformis* habitat included.

mixed-conifer forests includes gene conservation and studies of genecology and population genetics. Some of our other research directions not addressed here are regeneration ecology, silvics, sustainable management, and prediction of the future distribution of the species using landscape genetics and climate change modeling. Understanding ecophysiological and adaptive processes of this species will help inform genetic and silvicultural approaches to decrease forest tree vulnerability and increase adaptive capacities in the face of disturbances and a changing climate (Bower et al. 2011; Chmura et al. 2011).

GENE CONSERVATION

Our seed collections throughout the range of *P. strobiformis* in the Southwest began in 2012. Although

our primary goal was to archive seed for the U.S. Department of Agriculture, Forest Service (USDA FS) Gene Conservation program (Mangold 2011), we have collected enough additional seed to support research and management projects. Sites were identified using the known range of *P. strobiformis* in Arizona and New Mexico in conjunction with information about local cone crop production in a given year. At each site, we collected cones from up to five trees separated by a minimum distance of 200 ft (61 m) and recorded major overstory and understory species, presence of *Ribes* shrubs (one of the key secondary hosts for WPBR), slope, and aspect (see Schoettle et al. 2011). Each collection tree was georeferenced and tagged at breast height (1.37 m) for relocation purposes; we also recorded total tree height, live crown proportion, diameter at breast height, presence and severity of WPBR

Table 1—*Pinus strobiformis* seed collection sites and year of collection(s) across National Forests in the Southwest. Collections from Indian Reservations not shown.

Collection year(s)	Location	District	State	Gene conservation sites
2012-2014	Coconino National Forest	Peaks	AZ	7
2012	Coconino National Forest	Mogollon Rim	AZ	2
2012, 2014	Apache-Sitgreaves National Forests	Black Mesa	AZ	3
2012	Apache-Sitgreaves National Forests	Springerville	AZ	1
2012	Apache-Sitgreaves National Forests	Alpine	AZ	1
2012	Apache-Sitgreaves National Forests	Lakeside	AZ	1
2012	Coronado National Forest	Safford	AZ	2
2013	Coronado National Forest	Santa Catalina	AZ	4
2014	Coronado National Forest	Sierra Vista	AZ	1
2013	Kaibab National Forest	Williams	AZ	1
2013	Gila National Forest	Reserve	NM	1
2014	Santa Fe National Forest	Jemez	NM	1
2013	Lincoln National Forest	Sacramento	NM	3
2014	Cibola National Forest	Magdalena	NM	2
2012, 2014	Cibola National Forest	Mt. Taylor	NM	2

infection, and insect infestation levels in cones and seeds for each collection tree. In areas where WPBR was present, we attempted to collect seeds from infection-free trees (putatively resistant), but seeds were also collected from trees with visible WPBR infections. To date, we have collected cones and seeds across much of the range of *P. strobiformis* in Arizona and New Mexico and along local elevational gradients where possible (fig. 1). Our seed collections for gene conservation represent eight national forests and two Indian Reservations (table 1). These collections consist of 111 trees (26 populations archived in the Gene Conservation program) from 2012 and 2013 and 59 trees (12 archived populations) from 2014. These are in addition to previous collections of 88 families and 3 bulk populations of *P. strobiformis* in USDA FS Region 3 (southwestern United States; Sniezko et al. 2011).

GENECOLOGY RESEARCH

To properly manage *P. strobiformis* in the face of climate change and WPBR, adaptive traits and responses to stressors must be quantified from populations across the range; these are considered key objectives in successful restoration efforts in other species of threatened

five-needle pines (Bower et al. 2011; Hamlin et al. 2011). There is relatively little information about population differentiation of quantitative traits in *P. strobiformis* (but see Steinhoff and Andresen 1971). With known family and population variation in quantitative traits such as phenology, morphology, and drought tolerance, we can infer adaptation to geographic or climatic gradients, quantify heritability, and reduce risk of seedling maladaptation in seed transfer and restoration efforts (Bower and Aitken 2008; Bower et al. 2011). Because outplanting artificial regeneration with natural genetic resistance is a realistic option for managers, the risk of seedling maladaptation to new sites must be quantified with common garden studies (Bower and Aitken 2008; Keane and Schoettle 2011; Schoettle and Sniezko 2007).

We began a greenhouse common garden study in Flagstaff, Arizona, in spring 2012 with the specific objectives to determine family, population, and geographic variation in quantitative traits (growth, phenology, and the tolerance to low water treatments) of *P. strobiformis*. We used seed from our collections, as well as donated seed from previous collections (Region 3 Forest Health Protection 1997–2008; Conklin et al. 2009; Sniezko et al. 2011), in a design

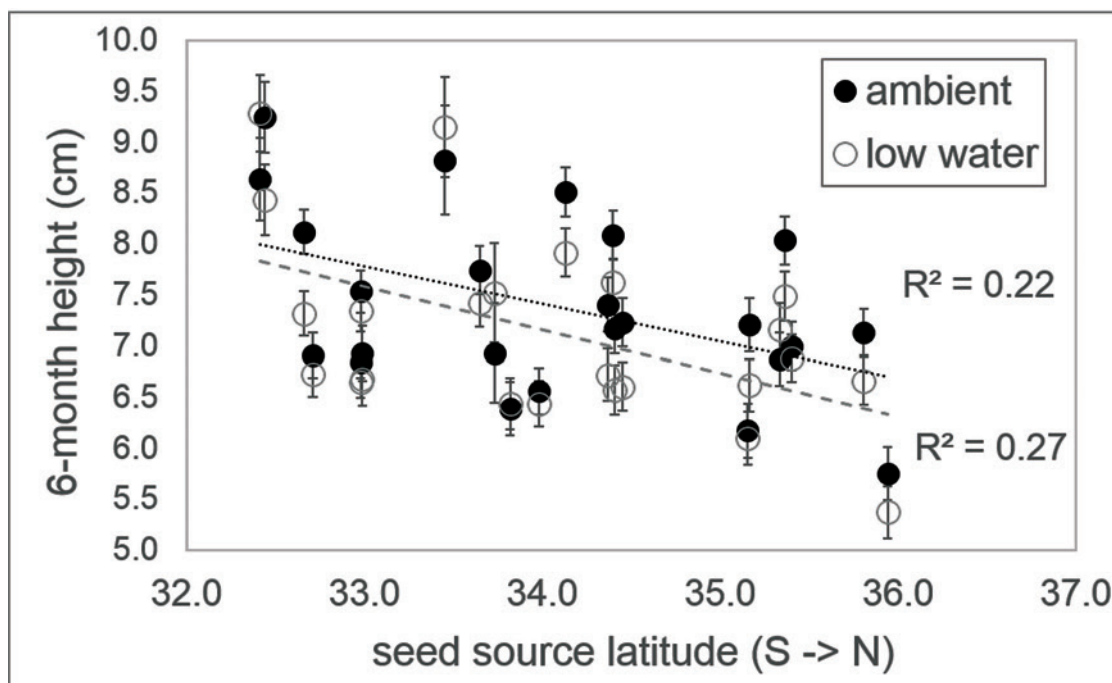


Figure 2—Relationship between 6-month-old seedling height means (± 1 standard error) and seed source latitude across two watering treatments in a greenhouse common garden study in Flagstaff, Arizona (ambient and low water). $N = 23$ populations, 2–15 families per populations, 18–36 seedlings per family.

that included 2 to 15 families (half or full-sibling offspring from a single mother tree) per population across 24 populations of *P. strobiformis*. Families were represented by 18 to 36 seedlings per family dependent upon germination rates, and half the seedlings from each family were subjected to low water treatments over 2 years. Growth, phenology, and physiological responses of all families, populations, and watering treatments were measured over two growing seasons. We hypothesized that (1) growth, phenology, and drought tolerance variation would be small within populations but large between populations because of the isolated, disjunct nature of *P. strobiformis* populations; (2) population seedling growth and phenology would parallel seed source climate (e.g., seedlings from source climates with fewer frost-free days would end growth earlier; seedlings from warmer source climates would grow larger); and (3) growth and survival under drought conditions would also parallel seed source climate.

Preliminary results indicated that growth trait population means such as 6-month height, number of branches (potential allocation to growth processes other than height), and growth initiation (phases of bud and leaf development) were well differentiated and appeared to correlate weakly with seed source geographic and climatic clines (fig. 2, $R^2 = 0.22$ and 0.27 for ambient and low water seedlings, respectively), indicating local adaptation of populations for these traits, which will be evaluated with reciprocal transplant gardens in the future. Overall, populations from southern sources grew taller than populations from northern sources (fig. 2). These patterns continued in the second growing season; population means of 14-month-old seedling heights were also weakly correlated with seed source latitude and southern populations grew larger than northern populations (data not shown; $R^2 = 0.23$ and 0.21 for ambient and low water treatments, respectively). Latitude was a stronger correlate than elevation for all growth traits measured in the first season. No

genotype-by-environment effects were observed in the first season for any measured trait and analyses are ongoing. Relationships between quantitative traits and climate variables are also being investigated.

ONGOING AND FUTURE RESEARCH DIRECTIONS

We are expanding the initial common garden from the greenhouse to the Southwest Experimental Garden Array (SEGA), a series of common gardens across an elevation gradient in northern Arizona. The first trial garden was planted in fall 2014 with 170 families from 44 populations (fig. 3). Many of these families are also included in disease resistance screening trials at the USDA FS Dorena Genetic Resource Center (DGRC, Cottage Grove, Oregon, USA) in a project funded by the USDA FS Forest Health Protection Special Technology Development Program (R3-2014-02). There are 39 populations of *P. strobiformis* represented by 154 families growing at DGRC slated to be

inoculated in fall 2015 for long-term testing of partial resistance mechanisms to *C. ribicola*. A small proportion of these populations were inoculated in fall 2014 for shorter term major gene resistance tests. We are developing two outplanting sites on national forest and tribal land in Arizona and New Mexico to test the long-term durability of resistant seedlings in the field, and families to be included in the outplanting will be chosen following early results from resistance trials.

We are also investigating population genetics across the entire range of *P. strobiformis*, beginning with 70 populations across the southwestern United States and Mexico. In Mexico, foliage was collected from 19 populations and 169 individuals in the Sierra Madre Occidental and Trans Mexican Volcanic Belt in May 2014, with additional foliage obtained from the botanical collection at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR, Interdisciplinary Research Center for Integrated Regional Development), Durango (fig. 4).



Figure 3—Initial common garden planting at The Arboretum, Flagstaff, Arizona. Site is part of the Southwest Experimental Garden Array. Six-month-old seedlings were planted in early October 2014.

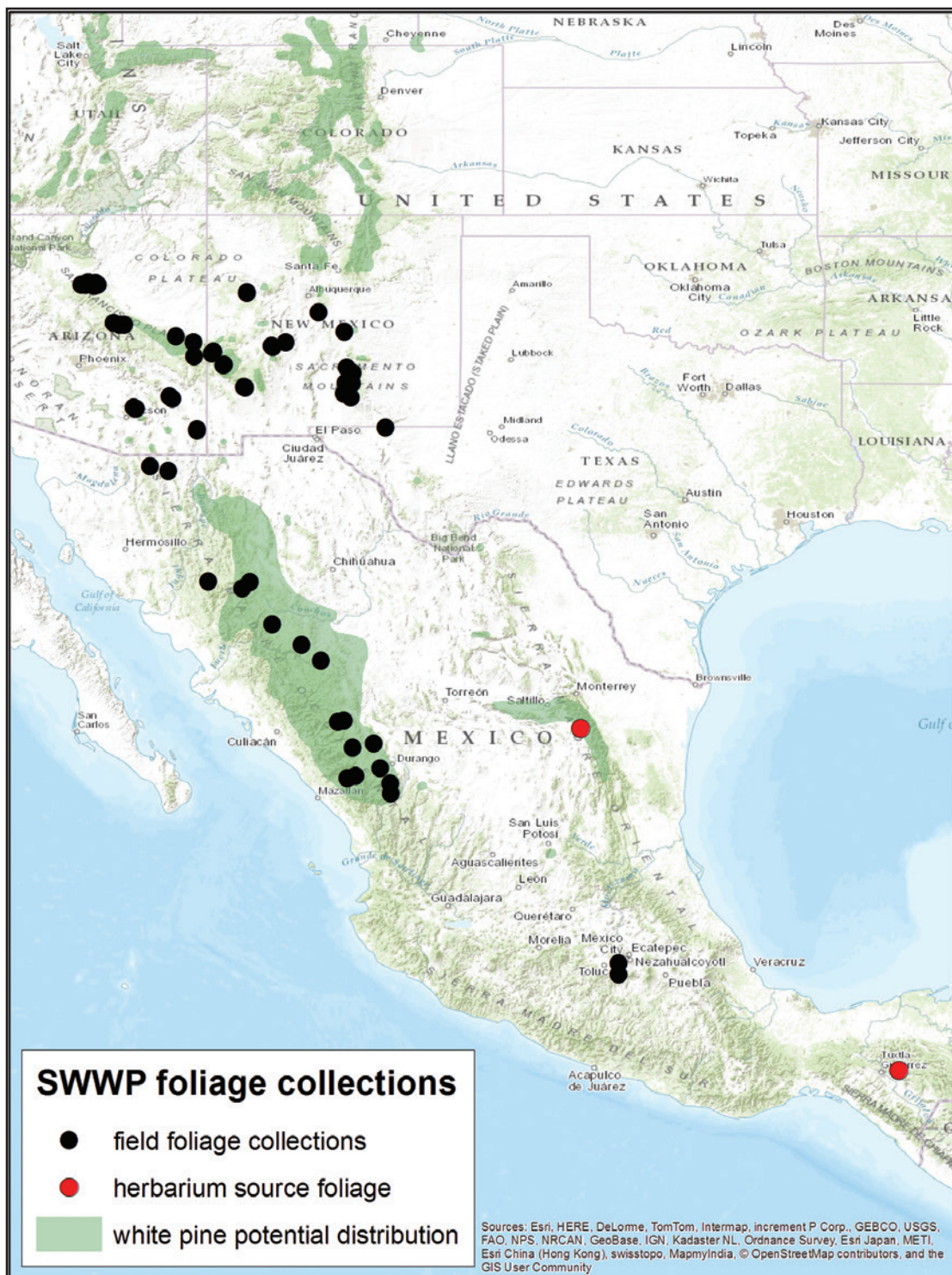


Figure 4—*Pinus strobiformis* foliage collection sites across the Southwest United States (Region 3) and northern Mexico for use in restriction-site associated DNA sequencing (RADSeq) to identify single nucleotide polymorphisms across the genome.

Collections included one population of *P. strobiformis* var. *stylesii* from Sierra Madre Oriental and one population of *P. ayacahuite* from Sierra Madre del Sur. We also collected foliage from 49 populations across

Arizona and New Mexico and from one *P. flexilis* (limber pine) population in southern Wyoming (fig. 4; Wyoming population not included in map). *Pinus flexilis*, *P. strobiformis* var. *stylesii*, and *P. ayacahuite*

populations were included to distinguish between closely related species and any hybridization that may occur.

Restriction-site associated DNA sequencing will be used to identify single nucleotide polymorphisms across the genome. We are conducting studies to understand the dynamics of gene flow, population structure, and hybridization to better understand the temporal and geographic severity of WPBR and climate change on *P. strobiformis*. Pines are well known for their long-distance gene flow and lack of incompatibility systems resulting in hybridization. It is therefore necessary to quantify the flow of genes related to disease resistance, drought tolerance, and other potentially adaptive traits across the entire range of *P. strobiformis* and hybrid zones with closely related species.

Future research plans will expand our seed collections across the range of the species (including more sites throughout Mexico), add three SEGA common gardens to the current experimental design, and add families and populations to disease resistance screening trials. Other ongoing and future objectives include determining linkages between adaptive traits and resistance frequencies, identifying the genetic bases for adaptive traits, and providing tools to better predict the potential distribution of *P. strobiformis* under future climate change. Our *P. strobiformis* research program combines basic and applied research using cross-collaborative efforts to sustain and conserve an important tree species in the southwestern United States and Mexico. Conserving the genetic diversity and defining management strategies to sustain *P. strobiformis* are critical in a changing climate and with the threat of WPBR. We envision a sustainable future for *P. strobiformis* with continued proactive and adaptive research and management.

ACKNOWLEDGMENTS

Funding for these projects has been provided by the USDA FS Forest Health Protection (FHP) Gene Conservation and Special Technology Development Programs, Northern Arizona University's (NAU's) Technology Research Initiative Fund, McIntire-Stennis appropriations to NAU and the State of Arizona, and

a Genes-to-Environment Fellowship to B. Goodrich. Seeds were donated for some projects by Dave Conklin (FHP Region 3), Detlev Vogler and Annie Mix (Institute of Forest Genetics), Anna Schoettle (Rocky Mountain Research Station), and Richard Sniezko (Dorena Genetic Resource Center). Amy Whipple (NAU Biology, Merriam Powell Center for Environmental Research) has been instrumental in genomics research and the future research program, including SEGA designs. Andrew Eckert (Virginia Commonwealth University) has also been instrumental in genomics research plans. We thank Brad St. Clair (Pacific Northwest Research Station) and James Jacobs (FHP Region 3) for reviews on an earlier version of this abstract and for their comments, which improved the publication. The following helped with lab and field work in the United States: Chris Erickson, John Benning, Shaughn Dugan, Sean Callahan, Hannah Stapleton, Stevia Morawski, Michael Scott, Travis Largent, Calvin Rogers, Danny DePinte, Gennaro Falco, Eric Vane, Adam Polinko, Connor Meehan, Ronnie Kylo, Kelsey Flathers, Adair Patterson, Tara Steadman, Marguerite Rapp, Julian Wilson, Nick Dolhyj, and Tanner Laird. In Mexico, foliage collections were facilitated by Socorro González-Elizondo (CIIDIR), Francisco Molina-Freaner (Universidad Nacional Autónoma de México [UNAM]), Christian A. Wehenkel (Universidad Juárez del Estado de Durango [UJED]), Aldair Flores (UNAM), Jorge Aguilar (Bosques de Árboles de Navidad), Jorge Noriega Villa (CIIDIR), Sergio Simental Rodríguez (UJED), José F. Martínez (UNAM), Gustavo Escobedo (Instituto Nacional de Astrofísica, Óptica y Electrónica), Valer Austin (Cuenca de los Ojos), José Manuel Pérez Cantú (Cuenca de los Ojos), Mario Cirett-Galán (Comisión Nacional de Áreas Naturales Protégidas [CONANP]), Isaias Ochoa (CONANP), Carlos Cohen (CONANP), Fernando Salazar Jiménez (Comisión Nacional Forestal), Ademir Flores, Yolanda Rentería, and José Molinar Rodríguez.

REFERENCES

- Alexander, B.G.; Ronco, F.; Fitzhugh, E.L.; [et al.]. 1984. A classification of forest habitat types of the Lincoln National Forest, New Mexico. Gen. Tech. Rep. RM-104. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 29 p.

- Bower, A.D.; Aitken, S.N. 2008. Ecological genetics and seed transfer guidelines for *Pinus albicaulis* (Pinaceae). *American Journal of Botany*. 95: 66–76.
- Bower, A.D.; McLane, S.C.; Echert, A.; [et al.]. 2011. Conservation genetics of high elevation five-needle white pines. In: Keane, Robert E.; Tomback, Diana F.; Murray, Michael P.; [et al.], eds. *The future of high elevation five-needle white pines in western North America: Proceedings of the high five symposium*. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 98–117.
- Chmura, D.J.; Anderson, P.D.; Howe, G.T.; [et al.]. 2011. Forest responses to climate change in the northwestern United States: Ecophysiological foundations for adaptive management. *Forest Ecology and Management*. 261: 1121–1142.
- Conklin, D.A. 2004. Development of the white pine blister rust outbreak in New Mexico. R3-04-01. Albuquerque, NM: U.S. Department of Agriculture, Forest Service, Southwestern Region. 17 p.
- Conklin, D.A.; Fairweather, M.L.; Ryerson, D.E.; [et al.]. 2009. White pines, blister rust, and management in the Southwest. R3-FH-09-01. Albuquerque, NM: U.S. Department of Agriculture, Forest Service, Southwestern Region. 16 p.
- Crookston, N.L.; Rehfeldt, G.E. 2008. Climate estimates and plant-climate relationships. Moscow, ID: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. <http://charcoal.cnre.vt.edu/climate/>. (05 October 2015).
- Fairweather, M.L.; Geils, B.W. 2011. First report of the white pine blister rust pathogen, *Cronartium ribicola*, in Arizona. *Plant Disease*. 95(4): 494.
- Fins, L.; Byler, J.; Ferguson, D.; [et al.]. 2002. Return of the giants: Restoring western white pine to the Inland Northwest. *Journal of Forestry*. 100: 20–26.
- Geils, B.W.; Conklin, D. A.; van Arsdell, E.P. 1999. A preliminary hazard model of white pine blister rust for the Sacramento Ranger District, Lincoln National Forest. Res. Note RMRS–RN–6. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 6 p.
- Hamlin, J., Kegley, A.; Snieszko, R. 2011. Genetic variation of whitebark pine (*Pinus albicaulis*) provenances and families from Oregon and Washington in juvenile height growth and needle color. In: Keane, Robert E.; Tomback, Diana F.; Murray, Michael P.; [et al.], eds. *The future of high-elevation, five-needle white pines in western North America: Proceedings of the high five symposium*. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 133–139.
- Hawksworth, F.G. 1990. White pine blister rust in southern New Mexico. *Plant Disease*. 74: 938.
- Looney, C.E.; Waring, K.M. 2012. Patterns of forest structure, competition and regeneration in southwestern white pine (*Pinus strobiformis*) forests. *Forest Ecology and Management*. 286: 159–170.
- Keane, R.E.; Schoettle, A.W. 2011. Strategies, tools, and challenges for sustaining and restoring high elevation five-needle white pine forests in western North America. In: Keane, Robert E.; Tomback, Diana F.; Murray, Michael P.; [et al.], eds. *The future of high-elevation, five-needle white pines in western North America: Proceedings of the high five symposium*. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 276–294.
- Kinloch, B.B.; Dupper, G.E. 2002. Genetic specificity in the white pine-blister rust pathosystem. *Phytopathology*. 92: 278–280.
- Looney, C.E.; Waring, K.M. 2012. Patterns of forest structure, competition and regeneration in southwestern white pine (*Pinus strobiformis*) forests. *Forest Ecology and Management*. 286: 159–170.
- Looney, C.E.; Waring, K.M. 2013. *Pinus strobiformis* (southwestern white pine) stand dynamics, regeneration, and disturbance ecology: A review. *Forest Ecology and Management*. 287: 90–102.
- Mangold, R.D. 2011. The U.S. Forest Service's renewed focus on gene conservation of five-needle pine species. In: Keane, Robert E.; Tomback, Diana F.; Murray, Michael P.; [et al.], eds. *The future of high-elevation, five-needle white pines in western North America: Proceedings of the high five symposium*. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 151.
- Moreno-Letelier, A.; Piñero, D. 2011. Phylogeographic structure of *Pinus strobiformis* Engelm. across the Chihuahuan Desert filter-barrier. *Journal of Biogeography*. 36: 121–131.
- Schoettle, A.W.; Snieszko, R.A. 2007. Proactive intervention to sustain high elevation pine ecosystems threatened by white pine blister rust. *Journal of Forest Research*. 12: 327–336.

- Schoettle, A.W.; Goodrich, B.A.; Klutsch, J.G.; [et al.]. 2011. The proactive strategy for sustaining five-needle pine populations: An example of its implementation in the southern Rocky Mountains. In: Keane, R.E.; Tomback, D.F.; Murray, M.P.; [et al.], eds. The future of high-elevation, five-needle white pines in western North America: Proceedings of the high five symposium. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 323–324.
- Snieszko, R.A.; Mahalovich, M.F.; Schoettle, A.W.; [et al.]. 2011. Past and current investigations of the genetic resistance to *Cronartium ribicola* in high-elevation five-needle pines. In: Keane, Robert E.; Tomback, Diana F.; Murray, Michael P.; [et al.], eds. The future of high-elevation, five-needle white pines in western North America: Proceedings of the high five symposium. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 246–264.
- Steinhoff, R.J.; Andresen, J.W. 1971. Geographic variation in *Pinus flexilis* and *Pinus strobiformis* and its bearing on their taxonomic status. *Silvae Genetica*. 20: 159–167.
- Sturrock, R.N.; Frankel, S.J.; Brown, A.V.; [et al.]. 2011. Climate change and forest diseases. *Plant Pathology*. 60: 133–149.
- Tomback, D.F.; Achuff, P. 2010. Blister rust and western forest biodiversity: Ecology, values and outlook for white pines. *Forest Pathology*. 40: 186–225.