

Proceedings of the 60th Annual Western International Forest Disease Work Conference

**October 8-12, 2012
Tahoe City, California**



***Proceedings of the 60th Annual Western International
Forest Disease Work Conference***

*October 8-12, 2012
Granlibakken
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Compiled by:

John Browning
Weyerhaeuser Forestry Research, Centralia, WA

and

Patsy Palacios
S.J. and Jessie E. Quinney Natural Resource Research Library
College of Natural Resources
Utah State University, Logan, UT

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Pete Angwin

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John Browning

Karen Hutton

Will Littke

Richard Reich

John Schwandt

Stephen Zeglen

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CHAIR'S OPENING REMARKS

Alex Woods¹

Welcome to amazing Lake Tahoe, California, on behalf of the WIFDWC Organizing Committee for the 60th annual Western International Forest Disease Work Conference. We have a full schedule for our program so I won't say much but I do have to say that it has been a real pleasure working with this year's organizing committee and I would like to thank them all now:

Local Arrangements: Phil Cannon and Bill Woodruff

Program Chair: Paul Hennon

Secretary: John Browning

Treasurer: Holly Kearns

Web Master: Judy Adams

We have had a series of conference calls over the past year to get things in order and each time it has seemed like we have had a good visit among friends. We are so happy to see so many attendees and probably no person is happier than Phil who has done a ton of work and a great job lining up this great facility. Thanks in particular to the graduate students and to the retirees for attending this year's meeting. To all of those that took part in the pre-meeting scavenger hunt, good on you. That was a great way to get these pathological things going!

Once again, welcome and have a great meeting and a great time renewing old friendships and starting new ones. Let's get this party started. (The following was not stated during the opening welcome but it is an honorable tradition to preserve).

A special part of the banquet program this year is dedicated to the recognition of several pillars of the WIFDWC family who passed away over the past year and a half.

Fields Cobb

University of California, Berkeley, CA.

Dick Parmeter

University of California, Berkeley, CA.

Bob Gilbertson

University of Arizona, Tucson, AZ.

Tom Laurent

USDA Forest Service, Juneau, AK.

Please think of these people and the contributions they made to the profession of forest pathology and to our larger society as a whole.

In: Browning, J. Comp. Proceedings of the 60th Annual Western International Forest Disease Work Conference; 2012 October 8-12; Tahoe City, CA. ¹BC Ministry of Forests, Lands and Natural Resource Operations, Smithers, BC.





KEYNOTE ADDRESS: A SUMMARY OF CURRENT TRENDS AND PROBABLE FUTURE TRENDS IN CLIMATE AND CLIMATE-DRIVEN PROCESSES IN THE LAKE TAHOE BASIN AND THE NEIGHBORING SIERRA NEVADA*

Hugh Safford¹

LOCAL TRENDS IN CLIMATE OVER THE PAST CENTURY

The data presented in this section are derived from the 98-year weather station record from Tahoe City, California, on the north shore of Lake Tahoe (WRCC 2008), and the annual State of the Lake Report published by the UC-Davis Tahoe Environmental Research Center (TERC 2008). Spatial data are also presented from the PRISM climate dataset, which extrapolates weather station records to the landscape for all years beginning in the late 19th century (Daly et al. 1994, PRISM 2010).

Temperature

Over the last century, mean annual temperature in the Lake Tahoe Basin (LTB) has risen by about two degrees F° (Figure 1). This trend is driven by a highly significant increase in mean minimum (i.e., nighttime) temperatures, which have risen by four degrees F since 1910. For the first time on record, the annual average of the monthly mean minima is now above the freezing point (Figure 1). Today the average is closer to six months, and the trend is strongly downward. The average number of days in a year on which the average air temperature remains below freezing has dropped by 27 days since 1910 (78 to 51; TERC 2008). The LTB rise in nighttime temperatures is higher than in most California locations and may be linked to the thermal mass of Lake Tahoe, whose surface waters have increased in temperature by one degree F in only the last 25 years (TERC 2008).

In: Browning, J. Comp. Proceedings of the 60th Annual Western International Forest Disease Work Conference; 2012 October 8-12; Tahoe City, CA. ¹USDA Forest Service, Pacific Southwest Region, Vallejo, CA. *The full-length version of this paper is presented in Safford, H.D., M. North, and M.D. Meyer. 2012. Climate change and the relevance of historical forest conditions. Pages 23-45, in: M. North, ed. Managing Sierra Nevada forests. General Technical Report PSW-GTR-237. USDA Forest Service, Pacific Southwest Research Station, Albany, CA. This includes additional information about climate change influences on wildfire, wildlife and hydrology.

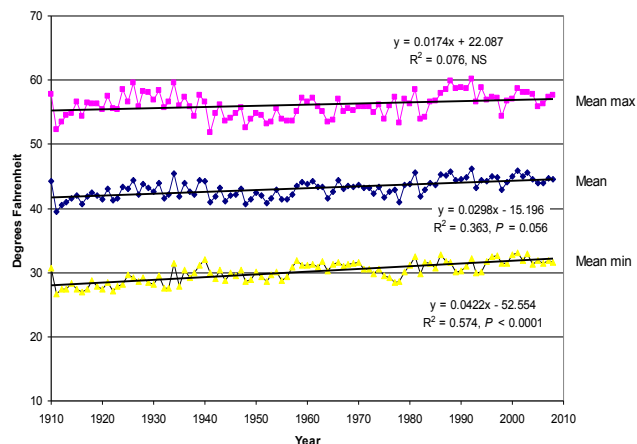


Figure 1. Annual mean, mean maximum, and mean minimum temperatures at Tahoe City, California, 1910-2008. Trend lines fit with simple linear regression, no transformations employed. Data from WRCC 2008.

Precipitation

The 98-year trend in LTB precipitation is shown in Figure 2. Average annual precipitation has risen by almost 7 in. per year over the period, but there is very high interannual variability, such that the value predicted by the regression line in Figure 2 is rarely representative of the actual annual mean. Of the months of the year, only August showed an even marginally significant increase in precipitation over the period of record ($R^2 = 0.034$, $P = 0.067$), with the average August precipitation rising from about 0.2 to about 0.4 in. (1 percent of annual precipitation). There were no significant increases in precipitation by season, and the distribution of precipitation across the year has remained similar through the record (WRCC 2008).

The 5-year coefficient of variation in annual precipitation is rising over time (Figure 3), which demonstrates that year-to-year variability in precipitation has increased over the course of the last century. Further evidence of high variability in recent annual precipitation sums can be seen in the last quarter-century of records: nine of the 20 wettest years have occurred since 1980, and two of the top three since 1995, but 2007 and 2008 are among the ten driest years on record.

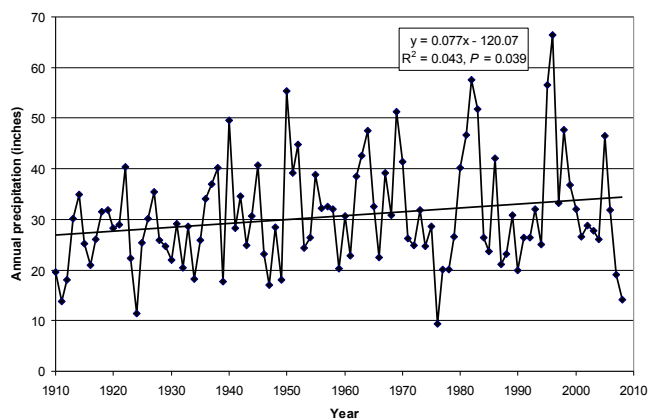


Figure 2. Mean annual precipitation at Tahoe City, California, 1910-2008. Data from WRCC 2008.

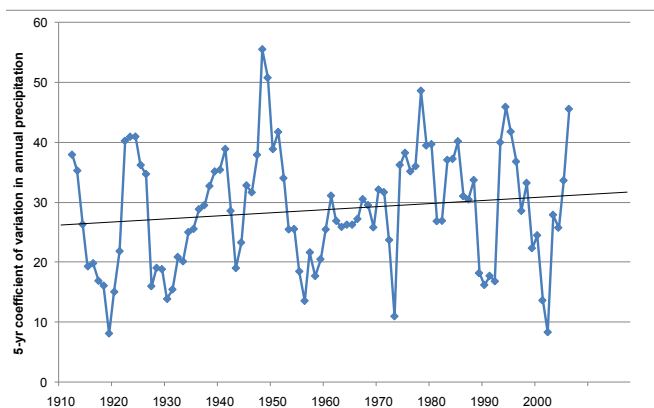


Figure 3. Five-year coefficients of variation in annual precipitation at Tahoe City, California, 1910-2008. Data from WRCC 2008.

Mean annual snowfall has not changed significantly over the last century (TERC 2008), but when combined with the precipitation trend, it is obvious that the proportion of precipitation falling as snow (vs. rain) is dropping. At the beginning of the last century, about 54 percent of precipitation fell as snow, today the average is about 34 percent. Streamflow data show that peak snowmelt in the LTB is occurring 2½ weeks earlier today than at the beginning of the 1960s, when the record began (TERC 2008). Snowpack measurements show a strong downward trend across northern California over the last ½ century, with the Sierra Nevada near Lake Tahoe experiencing decreases of >70 percent in snow water equivalent in many places (Figure 4).

The PRISM dataset shows that the area of the Sierra Nevada adjoining Lake Tahoe has experienced

substantial increases in both temperature and precipitation over the last ¾ century (Figure 5). This agrees with the trends from the Tahoe City station, but hides substantial variation among specific weather station sites.

REGIONAL TRENDS OVER THE LAST CENTURY LINKED TO CLIMATE CHANGE

Hydrology

Stewart et al. (2005) showed that the onset of spring thaw in most major streams in the central Sierra Nevada occurred 5-30 days earlier in 2002 than in 1948, and peak streamflow (measured as the center of mass annual flow) occurred 5-15 days earlier. During the same period, March flows in the studied streams were mostly higher by 5-20 percent, but June flows were mostly lower by the same amount; overall spring and early summer streamflow was down in most studied streams. Rising winter and spring temperatures appear to be the primary driver of these patterns (Stewart et al. 2005). Coats (2010) examined the shift in snowmelt timing in the Lake Tahoe Basin between 1972 and 2007 and found that the timing of the spring snowmelt peak occurred about two weeks earlier in 2007 than in 1972.

Forest Fires

Data on forest fire frequency, size, total area burned, and severity all show strong increases in the Sierra Nevada over the last two to three decades. Westerling et al. (2006) showed that increasing frequencies of large fires (>1000 acres) across the western United States since the 1980s were strongly linked to increasing temperatures and earlier spring snowmelt. The Sierra Nevada was one of two geographic areas of especially increased fire activity, which Westerling et al. (2006) ascribed to an interaction between climate and increased fuels due to fire suppression. Westerling et al. (2006) also identified the Sierra Nevada as being one of the geographic regions most likely to see further increases in fire activity due to future increases in temperature. Miller et al. (2009) showed that mean and maximum fire size, and total burned area in the Sierra Nevada has increased strongly between the early 1980s and 2007. Climatic variables explain very little of the pattern in fire size and area in the early 20th century, but 35-50 percent of the pattern in the last 25 years. The mean size of escaped fires in the Sierra Nevada was about 750 acres until the late 1970s, but the most recent ten-year

average has climbed to about 1100 acres. Miller et al. (2009) also showed that forest fire severity (a measure of the effect of fire on vegetation) rose strongly during the period 1984-2007, with the pattern centered in middle elevation conifer forests. Fires at the beginning of the record burned at an average of about 17 percent high (stand-replacing) severity, while the average for the last ten-year period was 30 percent. Miller et al. (2009) found that both climate change and increasing forest fuels were necessary to explain the patterns they analyzed.

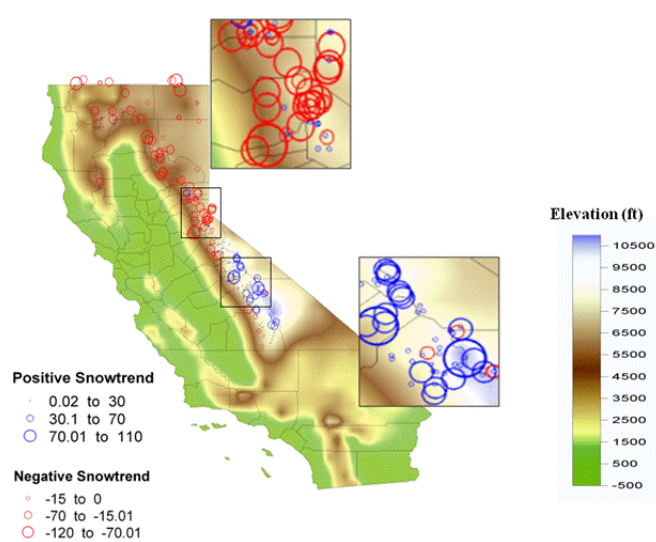


Figure 4. Trends in the amount of water contained in the snowpack (“snow water equivalent”) on April 1, for the period 1950-1997. Red circles indicate percent decrease in snow water; blue circles indicate increase in snow water. From Moser et al. (2009).

Forest Structure

Fire suppression has been practiced as a federal policy since 1935. Pre-Euroamerican fire frequencies in high elevation forests such as red fir (>50 years in most places) and subalpine forest (>100 years) were long enough that fire suppression has had little or no impact on ecological patterns or processes (Miller et al. 2009). Higher elevation forests are also much more remote, less likely to have economic uses, and are often protected in Wilderness Areas and National Parks, so impacts by logging or recreation use are minimal. Subalpine tree growth has been shown to be strongly influenced by higher precipitation and warm summers (Graumlich 1991). Long-term changes in stand structure in higher elevation forests are thus more likely to represent responses to changes in exogenous factors like climate.

In the early 1930s, the Forest Service mapped vegetation in the Lake Tahoe Basin and neighboring National Forests, and sampled thousands of vegetation plots (Wieslander 1935). Bouldin (1999) compared the Wieslander plots with the modern FIA inventory and described changes in forest structure. In red fir forest, Bouldin (1999) found that densities of young trees had increased by about 40 percent between 1935 and 1992, but densities of large trees had decreased by 50 percent during the same period.

In old-growth stands, overall densities and basal areas were higher, and the number of plots in the red fir zone dominated by shade-tolerant species increased at the expense of species like Jeffrey pine and western white pine. In old-growth subalpine forests, Bouldin (1999) found that young mountain hemlock was increasing in density and basal area while larger western white pine was decreasing. In whitebark pine stands, overall density was increasing due to increased recruitment of young trees, but species composition had not changed. Lodgepole pine appears to be responding favorably to increased warming and/or increased precipitation throughout the subalpine forest.

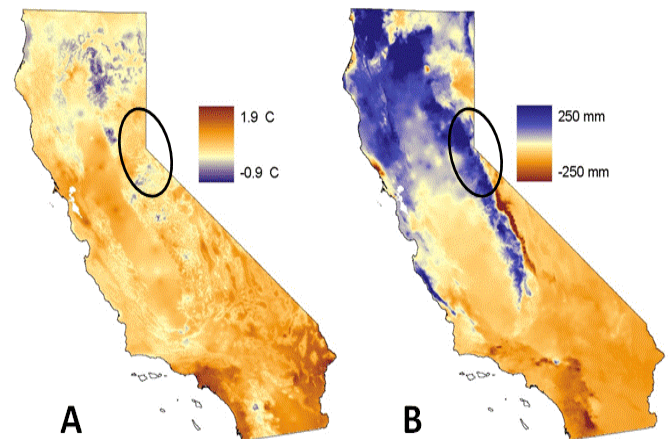


Figure 5. Spatial differences in mean annual temperature (A) and mean annual precipitation (B) between the 1930s and 2000s, as derived by the PRISM climate model. The LTBMU is found in the middle of the circled area. Both temperatures and precipitation have risen across most of the circled area, although precipitation has generally dropped east of the Sierra Nevada crest. Graphic courtesy of S. Dobrowski, Univ. of Montana.

Bouldin (1999) also studied mortality patterns in the 1935 and 1992 datasets. He found that mortality rates had increased in red fir, with the greatest increases in the smaller size-classes. At the same time, in subalpine forests, lodgepole pine, western white pine, and mountain hemlock all showed decreases in mortality. The subalpine zone was the only forest type Bouldin (1999) studied where mortality had not greatly increased since the 1935 inventory. This suggests that climate change (warming, plus steady or higher precipitation) is actually making conditions better for some tree species in this stressful environment. Dolanc et al. (2010) recently completed a study that resampled Wieslander plots in the subalpine zone between Yosemite National Park and the Lake Tahoe Basin. Corroborating Bouldin (1999), they found that growing conditions in the subalpine zone were probably better today than in the 1930s, as the density of small trees of almost all species had increased greatly in the 75 year period. Dolanc et al.'s (2010) direct plot-to-plot comparison also found that mortality of large trees had decreased density of the subalpine forest canopy, but the overall trend was for denser forests with no apparent change in relative tree species abundances.

Van Mantgem et al. (2009) recently documented widespread increases in tree mortality in old-growth forests across the west, including in the Sierra Nevada. Their plots had not experienced increases in density or basal area during the 15-40 year period between first and last census. The highest mortality rates were documented in the Sierra Nevada, and in middle elevation forests (3300-6700 ft.). Higher elevation forests (>6700 ft.) showed the lowest mortality rates, corroborating the Bouldin (1999) findings. Van Mantgem et al. (2009) ascribed the mortality patterns they analyzed to regional climate warming and associated drought stress. Comparisons of the 1930s Wieslander vegetation inventories and map with modern vegetation maps and inventories show large changes in the distribution of many Sierra Nevada vegetation types over the last 70-80 years (Figures 6a and 6b; Bouldin 1999, Moser et al. 2009, Thorne and Safford, unpub. data). The principal trends are (1) loss of yellow pine dominated forest, (2) increase in the area of forest dominated by shade-tolerant conifers (especially fir species), (3) loss of blue oak woodland, (4) increase in hardwood dominated forests, (5) loss of subalpine and alpine vegetation, and (6) expansion of

subalpine trees into previous permanent snowfields. Trends (4) through (6) appear to have a strong connection to climate warming, while trends (1) through (3) are mostly the product of human management choices, including logging, fire suppression, and urban expansion.

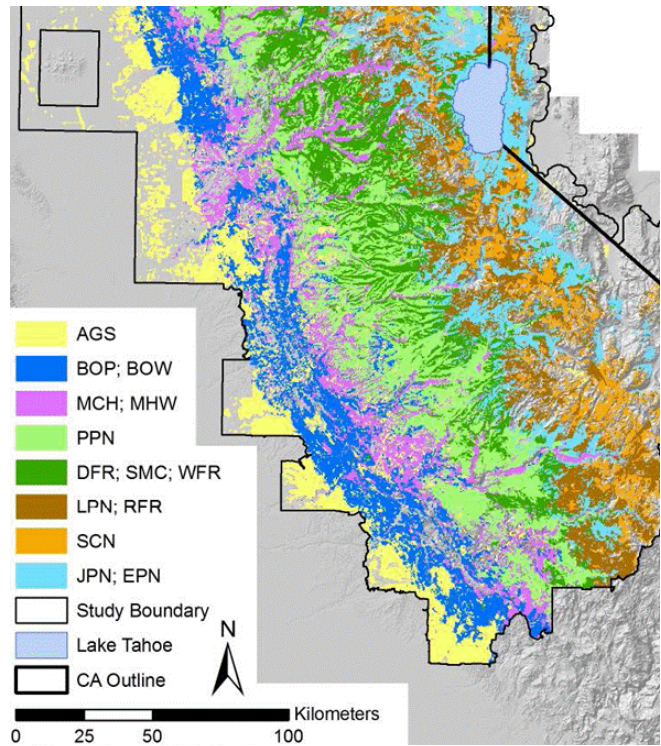


Figure 6. (A) Distribution of major vegetation types in the central and northern Sierra Nevada in the period 1932-1936. Mapped by the US Forest Service “Wieslander” mapping project. Maps digitized and vegetation types cross-walked to CWHR type by UC-Davis Information Center for the Environment. AGS = agriculture; BOP = blue oak/foothill pine; BOW = blue oak woodland; MCH = mixed conifer hardwood; MHW = mixed hardwood; PPN = ponderosa pine; DFR = Douglas-fir; SMC = Sierra mixed conifer; WFR = white fir; LPN = lodgepole pine; RFR = red fir; SCN = Subalpine conifer; JPN = Jeffrey pine; EPN = eastside pine.

FUTURE PREDICTIONS

Climate

Statewide models+

Relatively few future-climate modeling efforts have treated areas as restricted as the State of California. The principal limiting factor is the spatial scale of the General Circulation Models (GCMs) that are used to

simulate future climate scenarios. Most GCMs produce raster outputs with pixels that are 10,000's of km² in area. To be used at finer scales, these outputs must be downscaled using a series of algorithms and assumptions – these finer-scale secondary products currently provide the most credible sources we have for estimating potential outcomes of long-term climate change for California. Another complication is the extent to which GCMs disagree with respect to the probable outcomes of climate change. For example, a recent comparison of 21 published GCM outputs that included California found that estimates of future precipitation ranged from a 26 percent increase per 1° C increase in temperature to an 8 percent decrease (Gutowski et al. 2000, Hakkarinen and Smith 2003). That said, there was some broad consensus: all of the reviewed GCMs predicted warming temperatures for California, and 13 of 21 predicted higher precipitation (three showed no change and five predicted decreases). According to Dettinger (2005), the most common prediction among the most recent models (which are considerably more complex and, ideally, more credible) is temperature warming by about 9° F by 2100, with precipitation remaining similar or slightly reduced compared to today. Most models agreed that summers will be drier than they are currently, regardless of levels of annual precipitation.

The most widely cited of the recent California-wide modeling efforts is probably Hayhoe et al. (2004). Hayhoe et al. (2004) used two contrasting GCMs (much warmer and wetter, vs. somewhat warmer and drier) under low and high greenhouse gas emissions scenarios to make projections of climate change impact for California over the next century. By 2100, under all GCM x emissions scenarios, April 1 snowpack was down by -22 percent to -93 percent in the 6,700-10,000 ft. elevation belt, and the date of peak snowmelt was projected to occur from 3 to 24 days earlier in the season. Average temperatures were projected to increase by 2 to 4 degrees F in the winter, and 4-8 degrees in the summer. Finally, three of the four GCM x emissions scenarios employed by Hayhoe et al. (2004) predicted strong decreases in annual precipitation by 2100, ranging from -91 to -157 percent; the remaining scenario predicted a 38 percent increase.

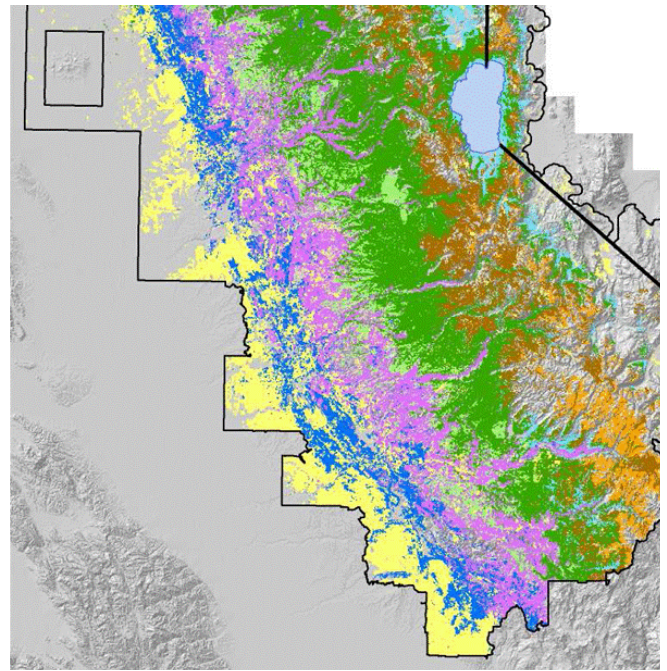


Figure 6. (B) Distribution of major vegetation types in the central and northern Sierra Nevada in 2000. Mapped by the US Forest Service Pacific Southwest Region Remote Sensing Laboratory. See figure 6 (A) for key and scale. The major patterns of change between 1934 and 2000 are: (1) loss of yellow pine (ponderosa and jeffrey pine) dominated forest; (2) expansion of shade tolerant conifers (dfr, wfr, smc); (3) loss of blue oak woodland; (4) increase in hardwood dominated forests; (5) loss of subalpine and alpine vegetation.

Vegetation

Lenihan et al. (2003, 2008) used a dynamic ecosystem model (“MC1”) which estimates the distribution and the productivity of terrestrial ecosystems such as forests, grasslands, and deserts across a grid of 100 km² cells. To this date, this is the highest resolution at which a model of this kind has been applied in California, but it is not of high enough resolution to be applied to the Lake Tahoe Basin as a unit. Based on their modeling results, Lenihan et al. (2003, 2008) projected that forest types and other vegetation dominated by woody plants in California would migrate to higher elevations as warmer temperatures make those areas suitable for colonization and survival. For example, with higher temperatures and a longer growing season, the area occupied by subalpine and alpine vegetation was predicted to decrease as evergreen conifer forests and shrublands migrate to higher altitudes (Figure 7). Under their “wet future” scenarios, Lenihan et al. (2003, 2008) projected a general expansion of forests in northern

California. With higher rainfall and higher nighttime minimum temperatures, broadleaf trees (especially oak species) were predicted to expand their distribution in many parts of the Sierra Nevada, and conifer-dominated forests were predicted to decrease in extent in the same areas. Under their “dry future” scenarios, Lenihan et al. (2003, 2008) predicted that grasslands would expand throughout the state, and that increases in the extent of tree-dominated vegetation would be minimal (Figure 7). An expansion of shrublands into conifer types was also predicted, due to drought and increases in fire frequency and severity (see below). Hayhoe et al. (2004) also used the MC1 ecosystem model to predict vegetation and ecosystem changes under a number of different future greenhouse gas emissions scenarios. Their results were qualitatively similar to the Lenihan et al. (2003, 2008) results.

Fire

The combination of warmer climate with higher CO₂ fertilization will likely cause more frequent and more extensive fires throughout western North America (Price and Rind 1994, Flannigan et al. 2000); fire responds rapidly to changes in climate and will likely overshadow the direct effects of climate change on tree species distributions and migrations (Flannigan et al. 2000, Dale et al. 2001). A temporal pattern of climate-driven increases in fire activity is already apparent in the western United States (Westerling et al. 2006), and modeling studies specific to California expect increased fire activity to persist and possibly accelerate under most future climate scenarios, due to increased growth of fuels under higher CO₂ (and in some cases precipitation), decreased fuel moistures from warmer dry season temperatures, and possibly increased thundercell activity (Price and Rind 1994, Miller and Urban 1999, Lenihan et al. 2003, 2008; Westerling and Bryant 2006). By 2100, Lenihan et al.’s (2003, 2008) simulations suggest a *c.* 5 percent to 8 percent increase in annual burned area across California, depending on the climate scenario (Figure 8). Increased frequencies and/or intensities of fire in coniferous forest in California will almost certainly drive changes in tree species compositions (Lenihan et al. 2003, 2008), and will likely reduce the size and extent of late-successional refugia (USFS and BLM 1994, McKenzie et al. 2004). Thus, if fire becomes more active under future climates, there may be significant repercussions

for old growth forest and old growth-dependent flora and fauna.

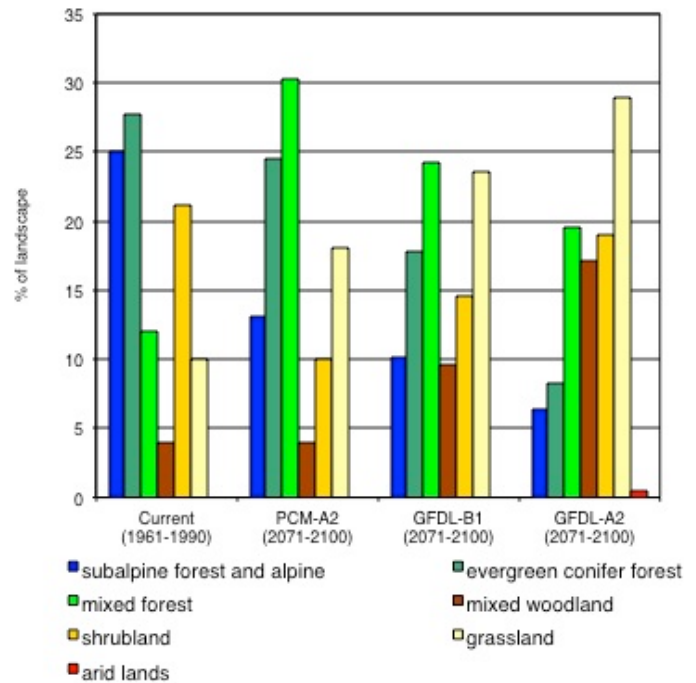


Figure 7. MC1 outputs for the Sierra Nevada Ecological Section, current vs. future projections of vegetation extent. The LTBMU is found within this Ecological Section. The GFDL-B1 scenario = moderately drier than today, with a moderate temperature increase (<5.5° F); PCM-A2 = similar ppt. to today, with <5.5° temp. increase; GFDL-A2 = much drier than today and much warmer (>7.2° higher) All scenarios project significant loss of subalpine and alpine vegetation. Most scenarios project lower cover of shrubland (including west side chaparral and east side sagebrush), due principally to increasing frequencies and extent of fire. Large increases in the hardwood component of forests are projected in all scenarios. Large increases in cover of grassland are projected for the Section, principally at lower elevations. Conifer forest decreases in cover under all scenarios. From Lenihan et al. (2008).

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2011 OUTSTANDING ACHIEVEMENT AWARD RECIPIENTS PRESENTATION

Ellen Goheen¹ and Susan Frankel²

“For Leadership in the science and practice of forest pathology and for critical contributions to the management of Sudden Oak Death”

Only you can prevent forest diseases!

I am honored and humbled to accept the 2011 Western International Forest Disease Work Conference (WIFDWC) Outstanding Achievement Award along with my friend and colleague, Ellen Goheen. In presenting the award, the Committee noted we are being recognized primarily for our contributions in response to the introduction of *Phytophthora ramorum*, the sudden oak death pathogen. Responding to the challenges of sudden oak death has altered my attitudes toward forest diseases: it inspires me to fight for the health of our forests with an urgency that I could not have previously imagined.

As a student, I assumed that foresters must thoroughly understand how forests grow and how to ensure they thrive despite human influence; after all, I was provided numerous, thick, detailed textbooks. Furthermore, professional forestry has prevailed for over a century, we have clear-cut millions of acres, constructed millions of miles of roads and diverted most of our rivers. But as I head towards the end of my career, I realize that despite all this collective experience, much work remains to be done for humans to learn how to co-exist with forests. Unfortunately, a decline in the number of forest pathologists is exacerbating this challenge to sustain forest health.

Over the past decade, there has been an uptick in forest pathology employment fueled by well-over \$100 million in support for sudden oak death pathogen monitoring, research, education, outreach and management. But now many of the laboratories that have been training students and gathering information are faced with funding shortfalls so each basic diagnostic capacity is being lost and our progress is threatened. It is critical

that forest pathologists share our accomplishments and concerns so society does not become complacent and assume their forests are protected from disturbance. We forest pathologists care and know more about tree diseases than anyone else and we need to make our concerns known.

CURRENT CHALLENGES

The response to sudden oak death, as detailed at suddenoakdeath.org, serves as a model for how to respond to a new forest pathogen but I wanted to highlight two current challenges.

1) It is difficult to identify the significance of new pathogens. *Phytophthora ramorum*'s symptoms include small, nondescript leaf spots that can easily be overlooked. Out in the forest, there are myriads of small defects on plants and it is not possible to recognize which are caused by new organisms that may be lurking with the potential to cause significant harm. Even when small patches of mortality are associated with a new organism, it is hard to predict how widespread damage may be if the pathogen spreads and becomes exposed to various environmental conditions.

2) Despite quarantines, there is inconsistent and limited reporting of monitoring results. The federal *Phytophthora ramorum* quarantine does not spell out exactly what information must be shared with horticultural or forestry professionals or the public. Each state retains the responsibility to report soil, water or wildland plant positive finds; reporting is, in some part, left to state discretion. It is not always in the best interest of a state to report a new find and information sharing beyond regulatory officials is limited. For federally quarantined forest pests, there is a need for clear national rules for reporting monitoring results and increased communication with the public.

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CONCLUDING OBSERVATIONS AND LESSONS LEARNED

Forest diseases are complex phenomenon, to continue to progress it is critical to understand the bigger picture: the organizational structure and culture of government, universities, land management organizations, state and federal regulatory bodies, and the values of local citizens. We need interdisciplinary studies and a broad understanding of ecology, entomology, soils, plant physiology, genetics, climate science, economics, social science and many other disciplines.

I would like to close with a list of lessons learned and an expression of my appreciation of the organizations and individuals who have supported me.

LESSONS LEARNED

1. Never use the term “gruesome” to describe tree mortality.
2. If you want to get people’s attention use the word “apocalyptic”.
3. Don’t call people when you are angry.
4. Be patient!!! Persist. Good guys usually win, eventually.
5. Tree pathogens have awesome capabilities.

THANK YOU...

Thanks to the USDA Forest Service, Pacific Southwest Research Station and the Pacific Southwest Region, Forest Health Protection for their support. Thanks to my teachers, mentors, colleagues, and to all that have assisted with the battle against the sudden oak death pathogen. A special thanks to Ellen Goheen for more than 30 years of friendship and camaraderie.





THE 2012 OUTSTANDING ACHIEVEMENT AWARD RECIPIENT JOHN SCHWANDT

**2012 Outstanding Achievement Award Committee
[Harry Kope, Bill Jacobi, and Paul Hennon]**

An award from the Membership of the Western International Forest Disease Work Conference

Each year the Outstanding Achievement Award Committee solicits nominations for individuals that have contributed significantly to the field of forest pathology in western North America. Responding to this call, the larger WIFDWC membership takes this opportunity to nominate one or more of their forest pathology colleagues to be acknowledged for their outstanding contributions.

This year the WIFDWC membership is awarding a long time member. The nomination is based on the member's career long contributions to forest pathology, particularly his work with white pine blister rust, his collaborations with natural resource professionals, his community involvement, tutoring and advocating for young people and promoting good urban forestry practices.

Quotes from nominating letters include support and testimonials of his forest pathology accomplishments, community advocacy of forestry and his outstanding character.

“He has devoted his energies and expertise to the applied management of white pine blister rust in young western white pine stands for over 20 years. It is no exaggeration to say that the current approach to, and extent of, western white pine management in the northern Rocky Mountains would not been the same without out his years of work.”

“We owe him a debt of gratitude for his administrative skills, collaborative interpersonal management style, and his record of accomplishment.”

“As further demonstration of his commitment to forest and tree health, he has been an active member of the Coeur d’Alene Urban Forestry Committee for over thirty years. In this role he is frequently called on to make tree health assessments, provide management recommendations for City green spaces and parks, and do develop standards for planting, replacement, pruning, and maintenance of City trees.”

“In addition to his notable accomplishments as a forest pathologist, I have always been impressed with how unfailing kind and thoughtful he has been with his work with others. He’s a teacher, always actively sharing information with forest managers, both in the classroom and in the field.”

“John’s passion is always evident and inevitably provides positive effects on the approach taken by others and the outcomes that are achieved. He never perceives anyone, no matter their age or level of education as ‘not important enough’ to receive his help and encouragement.”

Thus, the WIFDWC roll of Outstanding Achievement Award recipients has been increased by yet another outstanding forest pathologist. Congratulations to John for his excellent work and best wishes for his future endeavors.



**PANEL: ASSISTED MIGRATION AND FOREST DISEASES:
BOON OR LIABILITY?**





AN OVERVIEW OF SOME CONCEPTS, POTENTIALS, ISSUES, AND REALITIES OF ASSISTED MIGRATION FOR CLIMATE CHANGE ADAPTATION IN FORESTS

Louis R. Iverson¹, Matthew P. Peters¹, Stephen Matthews², and Anantha Prasad¹

INTRODUCTION

The climate has always been changing, but the rapid rate of climate change, as projected by the IPCC (2007) will likely place unique stresses on plant communities. In addition, anthropogenic barriers (e.g., fragmented land use) present a significant modern constraint that will limit the ability of species migration in responses to a changing climate. As such, managers are faced with four options that lay along a continuum when managing species in the face of climate change: (1) They can do nothing, and therefore allow existing landscapes to change without active intervention, accepting unknown or risky outcomes; (2) They can rely on passive resource management strategies to allow accommodation, such as linking existing preserves with corridors; (3) They can actively manage landscapes to preserve them as they are, thus create refuges. Such habitat management would include actions like preventing invasions, installing irrigation, and regulating biotic interactions; or (4) They can actively manage landscapes to convert them into something deemed more compatible with projected climatic conditions. This last example of management would include assisted migration. The specific risks and benefits of each of these actions will depend upon the magnitude of climate pressure, the context of the ecosystem and its landscape, and the goals of human decisions.

This paper describes some options on how to decide among the above choices, introduces assisted migration, and describes the possible ramifications associated with it. We then present one research approach to assist in locating and evaluating potential applications of assisted migration.

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DEFINITIONS

Assisted migration has been used synonymously in the literature with several terms, with slightly nuanced differences. We present here the definitions as published by a consortium of investigators on the topic (Schwartz et al. 2012):

- Translocation: Any intentional movement of a species from one location to another. (e.g., reintroducing wolves to Yellowstone National Park).
- Assisted Migration (AM): Introducing a species into a new location by bringing propagules or individuals and releasing them. (e.g., the movement of the tree *Torreya taxifolia* to North Carolina from its native range in Florida).
- Assisted Colonization: Assisted migration where the introduction is managed to ensure successful establishment. (e.g., translocated *Torreya* populations are carefully monitored and managed).
- Managed Relocation: The intentional act of moving species, populations, or genotypes to a location outside a target's known historical distribution for the purpose of maintaining biological diversity or ecosystem functioning as an adaptation strategy for climate change (e.g., introducing a butterfly into new habitat when current locations are likely to become unsuitable with climate change).

We also acknowledge two more terms, introduced by Pedlar et al. (2012) and revisited in the Johnson et al. paper of this volume, which add clarity to the discussion by making an important distinction:

- a. Species Rescue Assisted Migration: a means to rescue species threatened by climate change.
- b. Forestry Assisted Migration: aims to ensure that forests (often plantations) of widespread (often commercially valuable) tree species are established using seed sources that will be climatically adapted for the duration of the rotation. To be consistent with Johnson et al. (this volume), we will broaden this term to include using assisted migration to maintain ecosystem services, hereafter termed "Ecosystem Services AM".

THE DEBATE

The use of assisted migration has elicited controversy within conservation circles because balancing extinction risk against the potential negative impacts of managed relocation requires choosing between comparably unfortunate risks (Hoegh-Guldberg et al. 2008; Richardson et al. 2009; Schwartz et al. 2012). Opponents are concerned mostly because the placement of species outside their range may disturb native species and ecosystems when these “climate refugees” establish themselves in new environments; they cite many cases where intentional relocations resulted in a myriad of environmental issues (Davidson and Simkanin 2008; Ricciardi and Simberloff 2009; Seddon et al. 2009), like runaway invasions, that surface only after it is too late to turn back. Proponents point out that assisted migration is a key option to be available in the face of unprecedented global change (Sax et al. 2009; Schwartz et al. 2009; Minter and Collins 2010; Vitt et al. 2010). Concerns about species extinction, population extirpation, the loss of genetic diversity, and the maintenance of particular ecosystem services are paramount. For some species, conventional conservation strategies will not provide sufficient protection from future environmental change, and pressure to actively do something is likely to increase as the consequences of climate change become more apparent. Several groups have put together frameworks to evaluate risks and benefits related to assisted migration such that decision makers have solid approaches to use (Hoegh-Guldberg et al. 2008; Richardson et al. 2009; Seddon 2010; Lawler and Olden 2011; Schwartz et al. 2012).

The issue also provokes a number of legal issues that result from these unprecedented times of climate change. Camacho (2010) identified several key points that will be germane to the forest debate including a lack of clear jurisdiction precedence without regulatory mandates, especially for non-governmental assisted migration initiatives (e.g., in the *Torreya* example, a small group of individuals [the *Torreya* Guardians] moved the species). Another key topic raised by Camacho (2010) is the new paradigm that climate change brings to bear that natural systems can be dynamic (with climate change accelerating this notion) and traditional natural resource management must have the legal flexibility to respond. We must also recognize

that contemporary natural resource law’s fidelity to historic baselines, protecting preexisting biota, and shielding nature from human activity is increasingly untenable, particularly in light of climate change. More broadly, assisted migration illustrates how the natural resource organizations, laws and policies must be changed to better reflect a dynamic, globalized world with potential for major disruptions.

Finally, the choices we make come down to ethics. Do we prioritize to protect endangered species likely to lose habitat under climate change or do we focus on conserving native biota in situ? Do we manage ecological systems actively or leave nature wild and uncontrolled? Do we manage resources to promote their fitness under future conditions or work to preserve resources, as they exist today?

VALUE OF DISTINGUISHING ECOSYSTEM SERVICE AM FROM SPECIES RESCUE AM

One way to parse the debate is to subdivide assisted migration into Species Rescue AM and Ecosystem Services AM. As the names imply, the former is moving species to rescue them from extinction in the face of climate change, and this is the source of most of the uncertainty and controversy. The latter refers more to a traditional forestry approach aimed at maintaining high levels of productivity and diversity in widespread, commercially, socially, culturally, or ecologically valuable tree species (Gray et al. 2011; Kreyling et al. 2011). With Ecosystem Services AM, maintaining forest productivity and ecosystem services are the most obvious desired outcomes.

Given the broad distribution of most tree species, and the relatively short distances proposed for tree seed migration, Ecosystem Services AM typically involves transfers within or just beyond current range limits to locations where a population’s bioclimatic envelope is expected to reside within the lifetime of the planted population (Gray et al. 2011). Additionally, the introduction of genotypes to climatically appropriate locations may also contribute to overall forest health by establishing vigorous plantations across the landscape that are less susceptible to forest pests and diseases (Wu et al. 2005). If realized, such an outcome would help ensure the continued flow of ecosystem services provided by forests, such as wildlife habitat, erosion

prevention, carbon uptake, and many others (Kreyling et al. 2011). Thus, this form of assisted migration is much less controversial than the ‘rescue’ approach for species of special conservation concern. It is thus a viable tool at this time for adaptation to climate change in the forestry arena.

Pedlar et al. (2012) make the distinction between forms of AM based on intended outcomes, target species, movement logistics, potential risks, science-based feasibility, scope, cost, and practice. Ecosystem Services AM thus has several traits enabling the justification for AM, provided certain precautions are undertaken. When the discussion concerns trees, especially trees that are not necessarily rare or endangered, as is *Torreya taxifolia* (Schwartz 2005), it is often the case that planting trees in places where they previously did not occur has been done for centuries. The authors believe that, if practiced cautiously, and with the focus on moving species within or slightly beyond their current broadly-defined range margins to encourage ‘filling in’ of rarer occurrences, Ecosystem Services AM does hold promise as a relatively low risk climate change adaptation tool.

HOW MIGHT WE DECIDE WHETHER TO IMPLEMENT ASSISTED MIGRATION?

Land managers, through public participation, already are deciding among the four choices presented in the introductory paragraph. Such decisions will likely become more frequent and more involved as the rate of climate changes increases. Thus it is important to establish a set of approaches to choose from, and to include the choice of implementing assisted migration in some cases. Key to any approach is the following three elements:

1. Model potential outcomes in advance. We present an example below.
2. Evaluate the ecological impacts on both the target species and the recipient ecosystem, as well as the economic and social values influenced by management actions. This is accomplished through expert panels, modeling, experiments, and common sense evaluation.

3. Use a decision framework so that AM is only used with eyes wide open and often the last resort.

The authors endorse the decision framework presented by the Managed Relocation Working Group and published in Schwartz et al. (2012) and reiterated here without modification. They propose a set of key questions among four general themes that are central to creating a cohesive, broad-based general framework for decision making relative to proposed assisted migration actions. People are to answer each question as best possible and then weight them to arrive at a decision. Note that the economic and political considerations may override or modify many of the ethical and ecological questions in some situations.

Ethical Questions

1. What are the goals of conservation, and why do we value those goals?
2. Which conservation goals take ethical precedence over others and why?
3. What is the ethical responsibility of humans to protect biodiversity (genotypic, population, species, ecosystem)?
4. Is there an ethical responsibility to refrain from activities that may cause irreversible impacts, even if restraint increases the risk of negative outcomes?
5. How does society make decisions in consideration of divergent ethical perspectives?

Legal and Policy Questions

6. Do existing laws and policies enable appropriate managed relocation actions?
7. Do existing laws and policies inhibit inappropriate managed relocation actions?
8. Do the existing implementation policies of environmental laws provide the guidance for resource managers to fulfill their obligations for climate change adaptation?
9. What is the process for managers, stakeholders, and scientists to work collaboratively to make managed relocation decisions?
10. Who pays for managed relocation, including the studies needed to support an action, monitoring, and the outcomes of the management action?

Ecological Questions

11. To what extent do local adaptation, altered biotic interactions, no-analog climate space, and the persistence of suitable microhabitats within largely unsuitable landscapes mitigates the extinction risk (and managed relocation need) of species listed as vulnerable?
12. What evidence suggests that species are absent from climatically suitable locations because of dispersal limitations that could be addressed by managed relocation?
13. What are the limits of less dramatic alternatives to managed relocation, such as increasing habitat connectivity?
14. How well can we predict when management must address interacting suites of species rather than single species?
15. How well can we predict when relocated species will negatively affect host system species or ecosystem functioning (e.g., nutrient flux through food webs, or movement of individuals)?
16. How well can we predict the likelihood of a species' successful long-term establishment in light of a changing climate?

Integrated Questions

17. What are the priority taxa, ecosystem functions, and human benefits for which we would consider invoking managed relocation?
18. What evidence of threat (extinction risk, loss of function, loss of benefit to people) triggers the decision process?
19. What is adequate evidence that alternatives to managed relocation are unavailable and that the probability that managed relocation will succeed is adequate?
20. What constitutes an acceptable risk of harm and what are adequate assurances for the protection of recipient ecosystems?
21. Who is empowered to conduct managed relocation, and what is their responsibility in the event that the consequences are not those predicted?

AN EXAMPLE OF ASSISTING TREE SPECIES MIGRATION FOR FOREST ADAPTATION

Northern Wisconsin has served as a pilot landscape for a substantial amount of research on climate change and forest ecosystems as part of the Climate Change

Response Framework (www.climateframework.org). Northern Wisconsin forests have been the focus of a comprehensive climate change vulnerability assessment (Swanston et al. 2011), a large integrated effort to foster scientist - manager interaction (Brandt et al. 2012), and the development of an adaptation framework (Swanston and Janowiak 2012), all of which are intended to assist the region with forest management under climate change. The threats and vulnerabilities for many species and forest types have been changing in this area; many of these changes are directly or indirectly tied to the changes underway with climate, which is projected to change even more. For example by 2100, May–September (growing season) temperatures in this region are projected to increase substantially, leading to a wide-ranging set of impacts on forest ecosystems (Swanston et al. 2011). From this base of previous work in northern Wisconsin, we here initiate an effort to assess the feasibility and prioritization of assisted migration within this broader context.

We have evaluated 134 tree species for their current and future importance in the eastern United States, using our DISTRIB (Tree Atlas) modeling approach (Iverson et al. 2008; Prasad et al. 2009; Iverson et al. 2012). Briefly, in this approach, we model suitable habitat, as defined by those climatic, edaphic, and physiognomic conditions suitable for a particular species to occur. Using Random Forest modeling, 38 predictors (including 7 climate, 5 elevation, 9 soil class, 12 soil property, and 5 land use and fragmentation variables) are statistically correlated to species abundance derived from inventory data. The metric used for quantifying suitable habitat is summed importance values (IV) for any particular region of the eastern U.S. Thus, the area and the abundance of the species are accounted for, both now and potentially in the future. Our online website, www.nrs.fs.fed.us/atlas, provides a plethora of data for each of the 134 tree species as well as 147 bird species in the eastern U.S.

As part of the vulnerability assessment in northern Wisconsin (Swanston et al. 2011), 73 species were evaluated as being present currently or having suitable habitat in the future. The current range of one species, black oak (*Quercus velutina*), lies almost entirely to the south of this area, such that the species is almost exclusively located along the southern edge of vulnerability assessment region of northern Wisconsin.

As such, it is our candidate species to assess the feasibility for this species to move into the region, and we ask these two questions:

1. How might black oak move through a fragmented forest in northern Wisconsin under projected climate change?
2. How might this movement be augmented via assisted migration?

The migration potential for any species is related to both its source strength and sink strength. By source strength, we mean the propagule pressure - how many 'darts' can be sent out in front of the current boundary? This is related to the abundance of the species near its range boundary, and the distance the species must move to its new colonization site. Sink strength, on the other hand, is related to how receptive the new sites will be to the 'darts'. This is related to the future suitable habitat, as determined by amount of climate change, edaphic conditions, and fragmentation status. As we define it, a suitable sink must also be currently forested, so that a future suitable location for a colonizing tree must now have trees. As we model how black oak might move through the fragmented landscape in Wisconsin, the following steps are necessary.

1. Model Potential Changes in Suitable Habitat for Black Oak Under Two Scenarios of Climate Change

This initial modeling step provides the sink strength for the model. In the future, will the habitat be suitable for black oak? We assessed future habitat using the DISTRIB model for two scenarios of future climate – the Parallel Climate Model, B1 scenario (PCMlo - mild scenario (Washington et al. 2000)) and the Hadley CM3 model, A1fi scenario (Hadhi - harsh scenario (Pope 2000)). PCMlo is a mild warming scenario, while the Hadley A1fi is a much warmer scenario for Wisconsin. By assessing the range we can capture the bounds of modeled projected change; however, our planet is currently tracking and even possibly exceeding the warmest scenario (Canadell et al. 2007). The results show a substantial northward movement of suitable habitat into northern Wisconsin, especially under the Hadley scenario (Figure 1.) For the Hadley case, there

appears to be little ecological restriction for black oak suitable habitat in northern Wisconsin, but can it get there? To help answer this question, we need the additional steps (below) to prepare data and then use another model, SHIFT, which models migration potential over 100 years.

2. Create Defensible Range Boundary

Since the early 1970s, the standard bearer of range boundaries for tree species were the maps developed by E.L. Little (1971,1977), which are available online (through our group and the USGS) and are remarkable for their ability to portray the overall range extent for so many tree species across North America. However, in the 40+ years since Little was collecting data for these maps, there have been more sources of geographic distribution (most notably the impact of the US Forest Service Forest Inventory and Analysis (FIA) sampling (Miles et al. 2001), plus there may have been some actual distributional changes. The Little maps also tend to identify the absolute boundaries of the species (see boundary vs. abundance on Figure 1), whereas in some cases, we prefer a 'core boundary' to migrate from. Thus, we used a number of GIS tools in conjunction with FIA data and DISTRIB model outputs for current distribution to generate a 'Generalized Species Boundary' (Figure 2).

3. Map the Fragmented Forest

For modeling migration, we also needed a method to map the fragmented nature of the forest into which black oak must migrate. For this, we used the 2006 National Land Cover Data (Xian et al. 2009) and extracted the forest classes to create a forest-nonforest map at a resolution of 30 m. The 30-m cells were aggregated to 1 km, and if at least 10 percent of the cells were forest, the 1-km cell was deemed 'forested' for being able to accept propagules during migration. We then used the software 'GUIDOS' to determine 'core' from 'edge' forest (Vogt et al. 2007). This process produced a map showing the fragmented nature of the forests of the Wisconsin region (Figure 3).

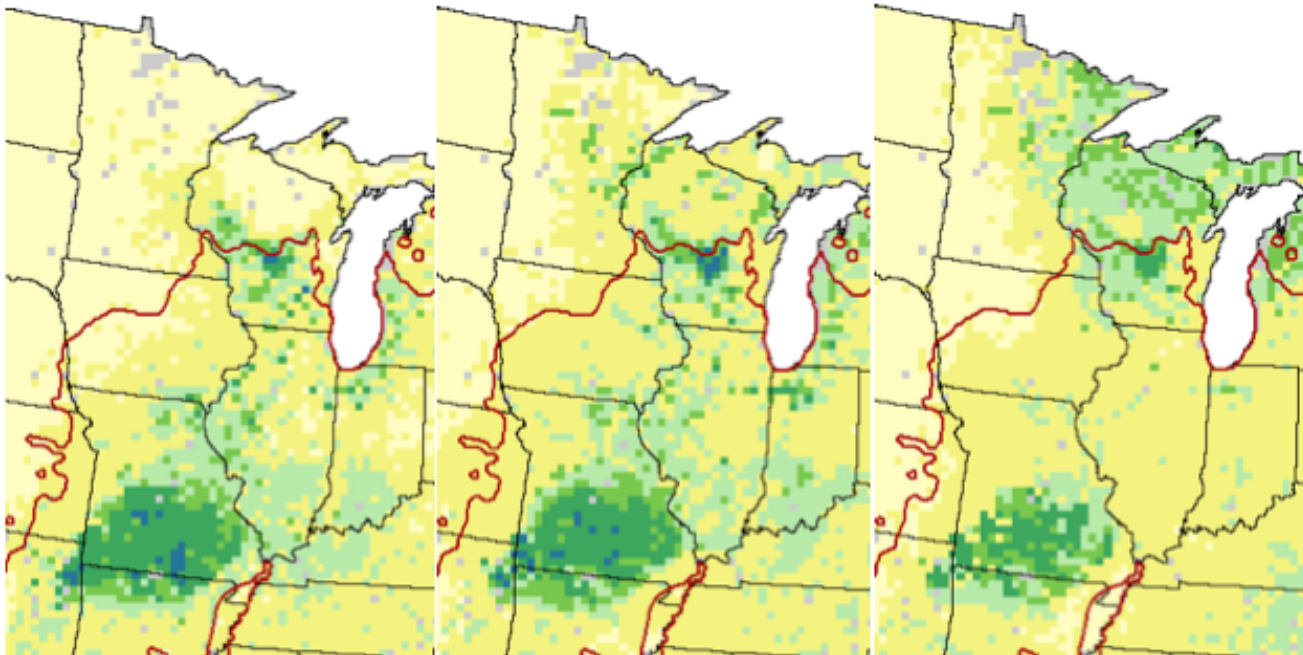


Figure 1. Current modeled importance of black oak (left) and projected change in suitable habitat under two scenarios of climate change: PCMIo (mild scenario; center) and Hadhi (harsh scenario; right). The brown line indicates the species range boundary for black oak according to Little (1971).

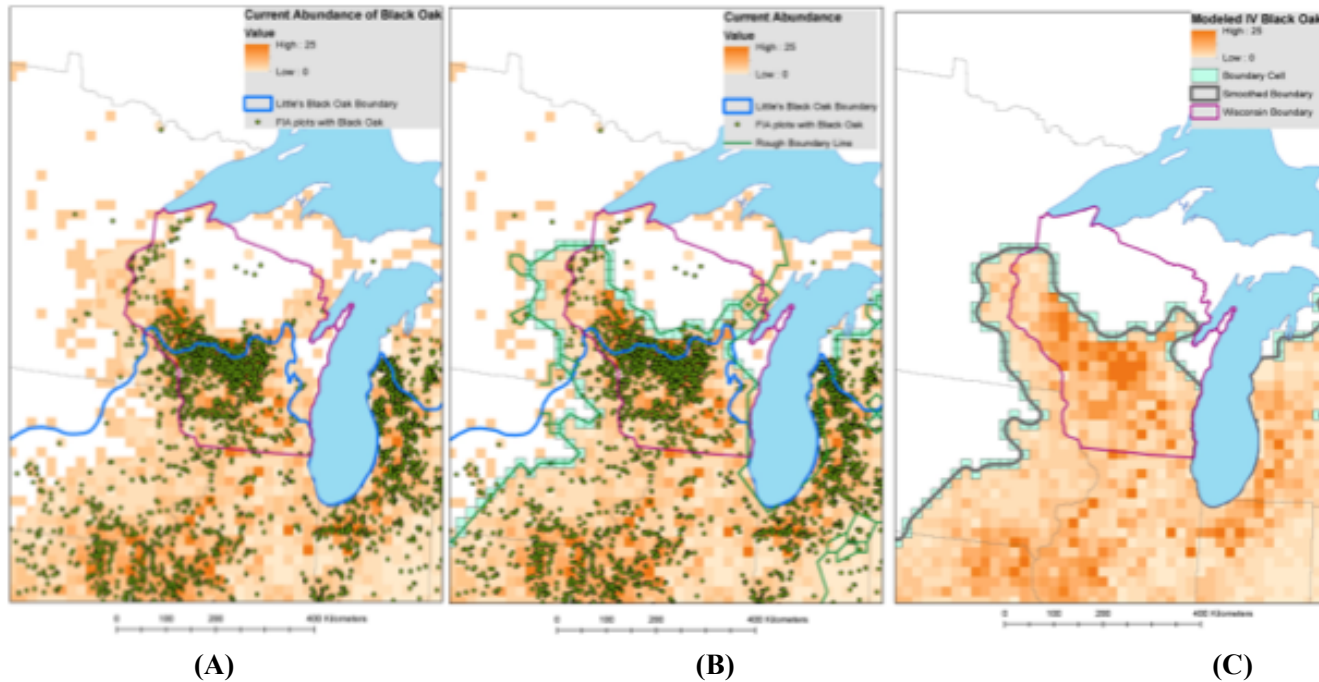


Figure 2. The basis and method to create a Generalized Core Boundary for black oak. **A)** Little's boundary does not adequately capture the current distribution of black oak as the current FIA plots show presence north of Little's boundary (blue line); **B)** an algorithm by S. Matthews identifies 'edge' pixels (in light blue) and rough boundary line (in green) based on the modeled current distribution, upon which some manual adjustments are made if needed to generalize the boundary further; **C)** the trimmed and smoothed boundary then is created to use in the SHIFT modeling.

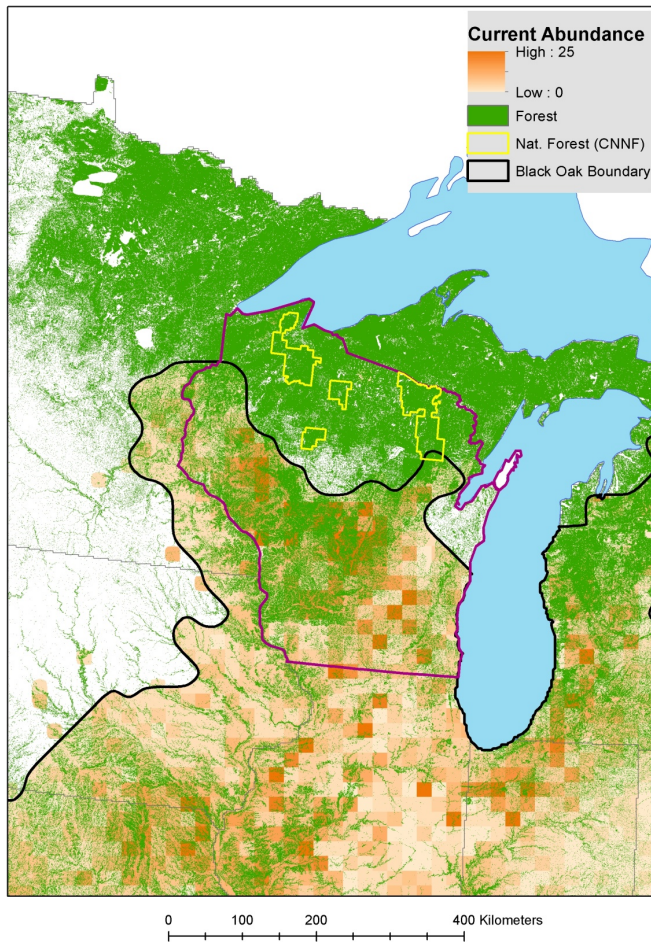


Figure 3. The Generalized Species Boundary line for black oak, with estimates of black oak abundance inside the boundary (current species range) and forest cover inside and outside the boundary. Yellow lines show the boundaries of the Chequamegon-Nicolet National Forest.

4. Model 100-Year Migration

Next was to run the SHIFT model, which uses simulation to estimate future colonization potential for black oak beyond the current range boundary over a 100-year period. The model is explained in previous publications (Iverson et al. 2004a, Iverson et al. 2004b) and fully developed in Prasad et al. (2013), but suffice it to say that it uses the source strength (black oak's abundance and distance from boundary edge) with sink strength (percent forest in the 1-km cell) to migration propagules. The rate of migration was calibrated to approximate 50 km/century, which is on the high end of migration rates estimated via paleoecologic data from the Holocene (Davis 1981). The output is the probability of a 1-km cell getting colonized in 100 years, over 2-4 generations depending on the species.

To constrain the SHIFT migration output with future suitable habitat as derived in step 1, the two model outputs were then combined to produce maps of probability of colonization where habitat will be suitable in 100 years under the 2 scenarios of climate change (Figure 4). This provides an estimate of potential migration success without human mediated assisted migration. To see the potential for that, we need to select appropriate locations for assisted migration to occur and rerun the SHIFT model.

5. Select and Add Locations for Assisted Migration

Selecting suitable locations for assisted migration is nontrivial, because one needs to prioritize and optimize over a number of criteria. For this example, we visually (via GIS) selected nine locations to assist (shown as red dots on Figure 5), based on:

1. Suitable habitat in future. The selected locations must contain suitable habitat in the future, preferably under both scenarios of climate change. This information is available from DISTRIB output (Figure 1).
2. Generally larger patches of forest. The larger patches of forest could be expected to more readily create a viable reservoir from the plantings, and thereby generate future expansion out from the assist. This information comes from the forest habitat map (Figure 3).
3. Promoting growth on/near the National Forest lands. Since the Chequamegon-Nicolet National Forest occupies some of the key forestland north of the current boundary of black oak (boundaries visible on Figure 3), and has substantial suitable habitat in the future, we modeled the creation of 'stepping stones' towards and within the National Forest boundaries as one example of targeted translocation efforts.

The selection of locations could be aided to a large degree by further GIS analysis, and that is our intention in later efforts. For example, the GUIDOS software (Vogt 2007) and others can help derive patches that are best connected to each other. GIS will be used to quantify the before and after assisted migration results. We present here only the visual results for example.

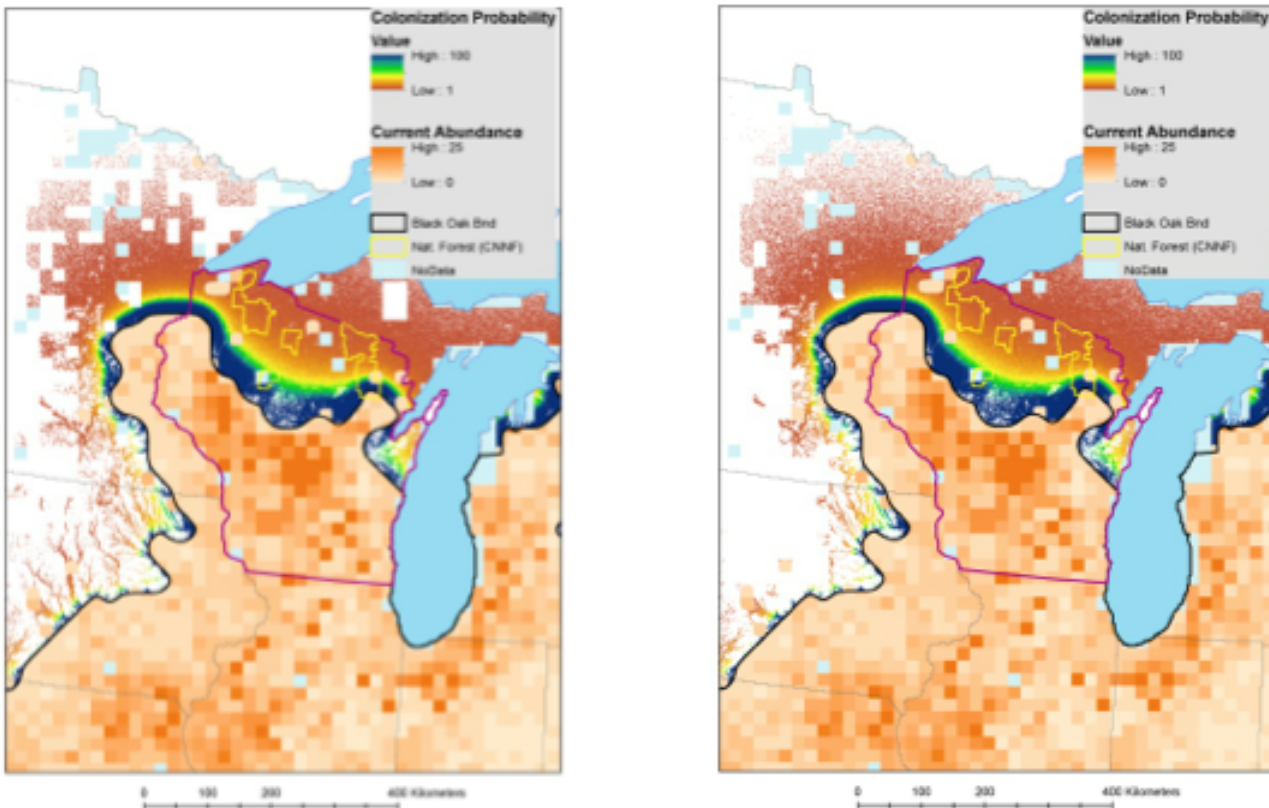


Figure 4. Probability of colonization over 100 years (at a rate of ~50 km/century) as overlaid on suitable habitat for 2100 with PCMIo (mild scenario; left) and Hadhi (harsh scenario; right). Also shown is modeled current abundance inside the black oak generalized boundary, with some peach-colored cells outside the current boundary being outlier cells with black oak currently present according to inventory data. If white, PCM or HAD do not project suitable habitat for black oak in 2100; no data cells refer to insufficient data for DISTRIB modeling.

6. Rerun SHIFT to Evaluate Potential Future Expansion After Assisting Migration

Following placement of the nine cells to accommodate the assisted migration, which assumes a low level of planting was accomplished throughout the 1-km cell, SHIFT was rerun with future habitat importance from DISTRIB providing the initial abundance, and to simulate 100 years of migration from the range boundary (which included some outliers present in the Little maps), and the new locations where assisted migration occurred (Figure 5). This map, when compared with the original SHIFT output, shows the distinct migration out from the outlying cells (where black oak was already present according to forest inventory data) but also a general rise in probability away from the current boundary because of the extra ‘darts’ generated by the outlying locations. Presumably, over time the outlying locations would amalgamate into regions of black oak presence.

In sum, the black oak presented is one example which provides an explorative, modeling framework for assessing and demonstrating the overall complexity of assisted migration approaches. Further research is needed to refine these methods and make them accessible to managers looking to plan or evaluate the potential to use assisted migration. Of course, additional research is also needed to better understand the genetics of any species under study for an assisted migration program, and the role of potential pests and pathogens in such a venture.

CONCLUSIONS

1. Considering Assisted Migration as a management option has merits, potential, and perhaps necessity, but care is advised!
2. Ecosystem Services (Forestry) AM has been underway for centuries, and carries fewer risks than Species Rescue AM; this distinction is useful.
3. Modeling experiments can aid in understanding how AM may work in the landscape.

4. However, a major research challenge remains to create distribution models that are relevant to, and sufficiently informative and scaled for, management decisions regarding translocations.

5. Included in this challenge is to better understand the role of pests and pathogens in the bigger AM picture, and thus it is vitally important for the forest disease and insect pest community to be engaged!

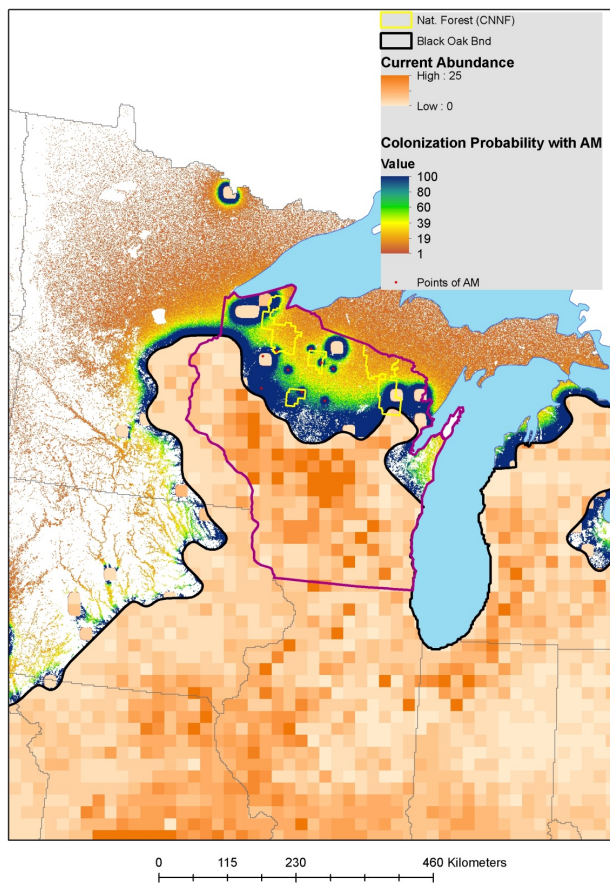


Figure 5. Probability of black oak colonization in 2100 following assisted migration of nine locations. Also included is the migration around current outliers.

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POLICY AND STRATEGY CONSIDERATIONS FOR ASSISTED MIGRATION ON USDA FOREST SERVICE LANDS

Randy Johnson¹, Sandy Boyce¹, Leslie Brandt², Vicky Erickson³, Louis Iverson⁴, Greg Kujawa¹, Larry Stritch¹, and Borys Tkacz¹

INTRODUCTION

Due to increased temperatures and shifts in precipitation patterns associated with climate change, bioclimatic zones that provide habitat for many species are expected to expand, contract, disappear, shift poleward, or move towards higher elevations (WGA 2008). Species will respond to changing climate and disturbance regimes individually, with some species moving quickly, others taking longer to move, and finally those incapable of keeping pace, which can lead to extinction (IPCC 2002; Chen et al. 2011).

Across the globe, species are already shifting their ranges in response to climate change (Parmesan and Yohe 2003; Gonzalez et al. 2010; Chen et al. 2011). Recent studies show that economically-important tree species in the eastern U.S. have already shifted their distributions in response to changes in climate, with some moving northward and others contracting (Woodall et al. 2009; Zhu et al. 2011). Some non-migratory butterflies in Europe have shifted north by 35-240 km over the 20th century (IPCC 2002). The Wildlife Corridors Initiative established by the Western Governors' Association found several bird species and hundreds of other plant and animal species in the western United States are also shifting their ranges several kilometers poleward or several meters upward in elevation per decade (WGA 2008).

Unfortunately, not all species have the ability to keep pace with a rapidly changing climate and disperse to newly suitable areas. Models of projected change in suitable habitat for tree species suggest that trees would need to migrate hundreds of feet to several miles per year to keep up with changes in climate (Iverson and

Prasad 2002). However, for widespread plant species the "unit" that needs to migrate is not the species per se, but populations within a species. Plant species with wide geographic ranges tend to have adaptive genetic variation patterned over the landscape that has resulted from natural selection of different traits for different environments where the species exist. The probability of survival for an individual of a specific population in a new location depends on whether it's genetic makeup 'pre-adapts' it to the new environment. The more 'fine-tuned' they are to their existing environment, the less able they are to exist elsewhere. In order to account for this variation in adaptive traits, it has been necessary to develop seed movement guidelines and breeding zones to ensure that reforestation and restoration activities result in adapted populations.

One option for overcoming a species', or population's, inability to migrate at the pace necessary to sustain itself under current and projected change in climate is the use of assisted migration. Assisted migration has been defined as the movement of species, populations, or genotypes to places outside the areas of their historical distributions to maintain biological diversity or ecosystem functioning with changing climate (Richardson et al. 2009; Schwartz et al. 2012). Assisted migration has been used synonymously with other terms such as managed relocation, assisted colonization, and managed translocation (Hunter 2007; McLachlan et al. 2007; Hoegh-Guldberg et al. 2008; Olden et al. 2011). Assisted migration may be motivated by a desire to (a) maintain genetic diversity, (b) protect species from extinction, (c) mimic dispersal interrupted by human habitat barriers, (d) maintain ecosystem functionality, or (e) maintain a population used in natural resource extraction (Schwartz et al. 2012). It has also been used to introduce desirable species (e.g., biological controls) where they have not existed previously. Regardless of the purpose, assisted migration is controversial.

In: Browning, J. Comp. Proceedings of the 60th Annual Western International Forest Disease Work Conference; 2012 October 8-12; Tahoe City, CA. ¹USDA Forest Service, Washington, DC. ²Northern Institute of Applied Climate Science, USDA Forest Service, St. Paul, MN. ³USDA Forest Service, Pacific Northwest Region, Pendleton, OR. ⁴USDA Forest Service, Northern Research Station, Delaware, OH.

Actions associated with assisted migration cover a wide variety of movements for a number of different purposes, ranging from moving a seed source¹ to another location within the species range in order to maintain ecosystem productivity, to moving a suite of species, or a community, outside of its historical range to prevent extinction. This paper examines two major categories of assisted migration that primarily impact management decisions on National Forest System (NFS) lands, **assisted migration to maintain ecosystem services** (*Ecosystem AM*) and **species rescue assisted migration** (*Species Rescue AM*); these definitions closely follow what is presented by Pedlar et al. (2012) for forest trees. These two categories differ in the types of species managed, management objectives, relative feasibility, and associated risks (summarized in Table 1; from Pedlar et al. 2012). Most discussions in the literature focus on species rescue and little on Ecosystem AM.

Moving a seed source outside its current population “range”, but within the range of the species, falls under the broad definition of assisted migration. In fact, even moving a genotype outside its current range falls within the broadest definition of assisted migration and is practiced regularly, since reforestation programs routinely use seed from parent trees (*in situ* or from seed orchards) that have never been on the restoration site.

Ecosystem AM aims to ensure that plantings of widespread species are established using seed sources that will be climatically adapted for decades to come. Maintaining climatic adaptation has been proposed to preserve forest health and productivity (O’Neill and Nigh 2011), which is necessary to maintain the continued flow of ecosystem services provided by forests, such as wildlife habitat, erosion prevention, timber, and carbon sequestration. Typically these plantings involve the use of seed sources outside of current seed zone delineations, but generally within a species’ range. *Species Rescue AM* is aimed at conserving species at risk of extinction in light of rapid climate change and/or other stressors.

This often involves moving a species outside its historical range to where conditions are thought to be better suited for the species than its current home sites. The Forest Service currently lacks specific guidance related to assisted migration. Current policies and guidance within the Forest Service are limited and do not distinguish between these two types of assisted migration. The risks and potential for success varies considerably between *Ecosystem AM* and *Species Rescue AM* and justifies the need for different policies (see Table 1, from Pedlar et al. 2012).

CURRENT FOREST SERVICE AM GOALS, PRINCIPLES, STRATEGIES, POLICIES, AND GUIDANCE

The mission of the Forest Service is to sustain the health, productivity and diversity of the nation’s forests and grasslands to meet the needs of current and future generations. The list of stressors affecting ecosystem health, productivity, and diversity continues to grow, with climate change direct and indirect effects poised to trump the entire list. With accelerating changes imminent, the agency is going to be hard pressed to sustain the health, productivity and diversity as the nation’s ecosystems quickly change. Waiting for the crisis may well result in triage management, or saving those species with a chance to exist and letting others perish. Is this the legacy of management the FS wants to have written about it in the history books? A better historical account should include well-reasoned goals, strategies, and actions designed to maintain the health, productivity and diversity of the nation’s ecosystems.

Existing FS Policies Forest Service AM policies are limited. However, some domestic and international research efforts include analysis and development of guidelines for assisted migration, such as the North American Forest Commission’s Forest Genetics Working Group Task 54: “develop guidelines for managed relocation of forest species and populations in response to climate change” (NAFC 2008). Another example is the Forest Tree Genetic Resource Management model of the Forest Genetics Council of British Columbia in Canada. Strategies include the use of managed relocation of tree species and seed sources as an adaptation tool (O’Neill 2008).

¹A seed source is seed collected from the locality in which the seedlings are to be grown.

Table 1. Comparison between forestry assisted migration (AM; referred to as Ecosystem AM in this document) and species rescue AM (from Pedlar et al. 2012).

Topic	Forestry AM	Species Rescue AM
Intended outcome	Maintain forest productivity and health under climate change	Avoid extinctions among threatened by climate change
Target species	Widespread, commercially valuable species	Species of conservation concern
Focal biological unit	Focuses on the movement of populations	Focuses on the movement of species
Movement logistics	Often within the current range of the species or within modest range extensions	Often well outside the natural range of species
Risks	Limited potential for creating an exotic invasive, limited potential to hybridize with new species, and limited potential to introduce disease to new populations or to other species	Some potential for creating an exotic invasive, some potential to hybridize with new species, and some potential to introduce disease to other species
Feasibility of science-based implementation	Provenance data for many commercial tree species, established seed procurement and storage methods, established best practices around plantation establishment, and autecology often well described	Provenance data not typically available, seeds not typically procured or stored, establishment best practices often not known, and autecology often well described
Scope	Potential to be employed across the millions of hectares that are regenerated annually in North America	Likely limited to suitable microsites
Cost	Adds little to existing forest regeneration costs (see the text for caveats)	Costs vary widely with the scope of the initiative
Practice	Already implemented in several regions	Very few known cases being implemented

Numerous environmental laws guide the agency to conserve and preserve the existing environment and, to the extent possible, restore ecosystem conditions (Marris 2008). Assisted migration changes the land management focus from past to future, and from small scale to large-scale solutions. It raises questions about laws intended to maintain the ecological status quo that may inhibit progress and requires modifying long-held views in conservation biology that are also supported, directly or indirectly, by federal laws and policies. Scientific and policy debates are expected to be controversial. There are a number of policies in the Forest Service Manual that impact assisted migration, including:

Reforestation Policy – FSM 2472.03

- Do not use seed and seedlings of exotic tree species or native species from an offsite source, except where:
 - a. Scientific studies have proven they are adaptable to the area in question.
 - b. Administrative studies or tests are being carefully planned with the cooperation or assistance from knowledgeable research scientists.

Genetic Resources Management – FSM 2475.03

- Use seedlings that are adapted to local climatic conditions. Use seedlings from distant sources only after successful performance in evaluation trials. Seedlings from distant sources may be used to accommodate projected changes in climate. Monitoring protocols should be established to track survival and performance of seedlings from distant sources. (*Note: this is new draft language from the FY12 manual revision*).

Native Plant Material Policy - FSM 2070.3

- Ensure genetically appropriate native plant materials are given primary consideration. (*Note: the policy defines genetically appropriate plants as being adapted to target site conditions with good establishment, vigor, and reproductive capabilities; sufficiently genetically diverse to respond and adapt to changing climates and environment conditions; unlikely to cause genetic contamination and undermine local adaptations, community interactions and function of resident native species within the ecosystem; not likely to become (not natural or inappropriate) invasive and displace other native species; and not likely to be a source of non-native invasive pathogens; likely to maintain critical connections with pollinators*).

- Select non-native plants as interim, non-persistent plant materials provided they will not hybridize with local species, will not permanently displace native species or offer serious long-term competition to the recovery of endemic plants, and are designed to aid in the reestablishment of native plant communities.

Management of Wildlife and Fish in Wilderness – FSM 2323.3

- Provide an environment where the forces of natural selection and survival rather than human actions determine which and what numbers of wildlife species will exist.
- Reintroduce wildlife species only if the species was once indigenous to an area and was extirpated by human induced events.

Threatened, Endangered, and Sensitive Plants and Animals: Experimental Populations – FSM 2671.43

- Experimental populations are those populations of threatened and endangered species so declared by the Secretary of the Interior, which are wholly separate geographically from naturally occurring populations of the same species. Experimental populations are exempt from the full protective measures of the Endangered Species Act of 1973, as amended, in order to encourage reintroductions of listed species and experimental approaches to accelerate recovery.
- All experimental populations shall receive the same treatment as "threatened" species.
- The Secretary of the Interior may issue regulations to allow for appropriate conditions and levels of "takings."
- Critical habitat is not declared for experimental populations.
- The Secretary of Interior may declare further that certain experimental populations are "nonessential" to the continued existence of the species.
- For the purposes of consultation requirements, nonessential experimental populations receive the same treatment as species proposed for listing. Consequently, the Forest Service must "confer" with the Secretary of the Interior or Commerce in accordance with requirements for proposed species (FSM 2671.45b).

In general, these statements imply that the only instance one should engage in assisted migration on an operational basis is when past scientific research supports success. In addition, untested assisted migration can take place if it is part of a research or administrative study. In all cases, monitoring is required. Presently, this limits operational assisted migration to only the handful of species that have provenance trial data available from longer-term field trials and those species where seed sources have been moved previously.

In terms of U.S. Forest Service strategy and guidance, both the USDA Strategic Plan for 2010-2015 and the agency's *National Roadmap for Responding to Climate Change* (USDA Forest Service, July 2010) encourage the use of practices that result in resilient landscapes and the need to conserve our genetic resources. Neither specifically addresses the specifics of assisted migration.

NFS geneticists and Research and Development (R&D) research geneticists recently completed a national white paper containing recommendations for adapting forest tree species to climate change (Erickson et al. 2012). The white paper identified a number of priority action items to facilitate implementation of assisted migration and other management recommendations aimed at enhancing forest resilience and resistance to changing climates. The premise is that assisted migration should only be done operationally when seed movement studies had been done for the specific species in question or when forest health, regeneration or productivity monitoring data indicate there are climate change related problems. The white paper encouraged the establishment of additional assisted migration trials. The principles emphasized in the document are in appendix 1. The general guidelines from this effort were:

- Starting point: consider species and local seed sources that have worked well in the past (locally adapted seed sources).
- If reforestation problems exist, expand local sources with germplasm better matched to the changed conditions. Emphasize genetic diversity, including the use of multiple species and diverse seed sources, and the maintenance of large populations with high connectivity and opportunity for migration of

adapted genes (via seed and pollen) in the direction of trending climates.

- Utilize a 10-20 year planning horizon for decision making (to minimize risks at the highly vulnerable seedling/sapling stage).
- Take high-risk actions (e.g., Species Rescue AM) over small areas on an experimental basis, or for genetic rescue of species and populations at imminent risk of extirpation.
- Take low-risk actions (moving a seed source within as species range) over larger areas.

POLICY NEEDS

The current lack of specific policies on assisted migration for commercially-important species, species targeted for use in restoration, and species of conservation concern leaves a gap in the ability of the Forest Service to make decisions on these important issues. Efforts are already underway to develop climate change adaptation strategies on our national forests, and assisted migration is a tool that may become necessary if we are to conserve species and maintain productivity under changing climatic conditions. Schwartz et al. (2012) suggest that federal agencies need to develop and adopt best practices that consider ethics, law, policy and ecology.

Present policy suggests that one should consider the success of a species/seed source in experimental trials before undertaking any assisted migration; this policy is conservative, and possibly rightly so since species distribution models (also called “climate envelopes”) have not always accurately or consistently predicted past and current range shifts (e.g., see Crimmins et al. 2011; Zhu et al. 2012). In addition, there is the concern that a translocated species may become invasive in its new environment or cause other problems such as introduction of novel insects or pathogens, or disruption of critical plant-pollinator connections (see Ricciardi and Simberloff 2009). The suggestion that translocation trials are kept small ensures that if a species or seed source becomes a problem there is the possibility of containment or eradication.

Assisted migration studies will help inform managers on the possibility of successfully moving a species or seed source, but this does not address all of the concerns

that are voiced in the literature (e.g., Richardson et al. 2009; Aubin et al. 2011; Schwartz et al. 2012). The literature suggests that any policy concerning assisted migration actions should undergo a thorough and transparent risk-benefit analysis before one makes a decision. Factors to be examined include:

- Success probability of the species/population being moved.
- Risk of the species becoming extinct with no action / probability of a stand becoming less productive with no action (or using local seed sources).
- Potential for the transplanted species/population to become invasive.
- Risks of moving invasive insects or pathogens along with transplanted species/population.
- Legal concerns around possible laws prohibiting movements.
- Ethical concerns and social acceptance.

Along these lines, Richardson et al. (2009) present a framework for evaluation that includes examining ecological and social criteria for four categories: focal impact, collateral impact, feasibility, and acceptability. This framework, or something similar, would be a useful tool to evaluate Forest Service assisted migration proposals. Pedlar et al. (2012) used a similar framework to compare Species rescue AM and Forestry AM (very similar to Ecosystem AM defined here) and demonstrated that there are much fewer risks involved in Ecosystem/Forestry AM than Species rescue AM (Table 1). Future policy must be realistic, as well as thorough; so that requirements are not so arduous that it would be impossible to proceed with appropriate management actions.

CURRENT FOREST SERVICE ASSISTED MIGRATION ACTIVITIES

Assisted migration activities within the Forest Service are limited and include:

- Many of the past and current provenance studies (very small scale Ecosystem AM).
- Operationally using seed from seed zones one elevation zone lower than planting site.
- Moving some southeastern conifer species north one seed zone.
- Using native cultivars in restoration whose original source was from a different seed zone or ecoregion.

CONCLUSIONS

Forest Service policy on assisted migration is minimal at present; only calling for scientific evidence that a move would be successful. For most species, such information is lacking and this may hinder proactive efforts to adapt NFS lands to projected changes in climate. In order for assisted migration to be socially acceptable, it will be necessary to expand policy to examine the multiple risks and benefits that could arise from the different types of assisted migration efforts that are being considered. The risks and benefits will vary depending on the type of assisted migration (Ecosystem AM or Species Rescue AM) and the species being considered.

For any policy, implementation can, and will, vary depending on who is doing the implementing. Factors that vary among people (and organizations) include:

- Overall objectives of the agency (company) and/or restoration project.
- Mindset / frame of reference. Some see management as a logical way to maintain the flow of ecosystem goods and services; others feel that nature will take care of itself and see management as an unwanted-intrusion into nature (Aubin et al. 2011). For example, Schwartz et al. 2012 makes the statement: *“The magnitude of projected climate change, however, suggests that humans may be forced to choose between the unfortunate alternatives of witnessing extinctions and intentionally manipulating species’ distributions in efforts to prevent extinction and maintain biodiversity.”* To many the choice is obvious, but the obvious choice can differ between any two people. In the case of the Forest Service, our mission is to sustain the health, diversity, and productivity of the Nation’s forests and grasslands to meet the needs of present and future generations; this implies the need to manage and not sit back and watch.
- Risk adverseness. Individuals differ in the amount of risk with which they are comfortable.

The Forest Service will have to weigh these factors when considering the best course of action for the Agency as it develops policies for assisted migration.

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APPENDIX 1

Principles and recommendations put forward by NFS and R&D geneticists working group (Erickson et al.) 2012.

PRINCIPLE 1: Genetically diverse and adapted seed and planting stock provide the foundation for healthy forests and ecosystems in the future.

Strategic Goal 1.1. Develop and deploy plant material that will be resilient to climate change.

Strategic Goal 1.2. Manage for uncertainty and adaptation through natural selection by placing an increased emphasis on genetic diversity (species and seed sources), as well as a diversity of silvicultural approaches across the landscape.

Strategic Goal 1.3. Consider climate change when determining plans and priorities for disease and insect resistance selection and breeding programs.

Strategic Goal 1.4. Create opportunities for rapid natural selection for species, habitats, and geographic areas with high observed or predicted potential for adverse impacts due to climate change.

PRINCIPLE 2: Gene conservation is key to preserving vulnerable species and populations for the future.

Strategic Goal 2.1. Preserve representative samples of species and populations threatened by climate change.

PRINCIPLE 3: Establishing partnerships will be more important than ever.

Strategic Goal 3.1. Support and expand internal and external partnerships that will improve our response to climate change.

The changes to current policy / activities:

Initiate new common garden and provenance tests for lesser known species and geographic areas.

Initiate assisted migration trials for key species.

Protect and maintain existing seed orchards and establish new orchards for priority species and geographic areas.

Enlarge and expand seed banks for reforestation/restoration and gene conservation.

Update seed management systems to provide maximum flexibility in an uncertain future.



CONSIDERING FOREST DISEASE IN ASSISTED MIGRATION STRATEGIES

Laura K. Gray¹, John H. Russell², Alvin D. Yanchuk³, and Barbara J. Hawkins¹

Assisted migration has quickly emerged as the leading climate change mitigation strategy within the forestry sector. For reforestation, the aim is to match locally adapted populations with their optimal climatic environments under anticipated future climates. While this practice addresses climatic mal-adaptation for tree species and populations, seed transfer decisions are primarily based on projected productivity and effectively ignore forest health concerns. Under the general principal that “warmer is better” for endemic pathogens, climate change may favor range expansion or increase the severity of these infectious agents (Dukes et al. 2009), which could result in unforeseen losses within plantations. For example, O’Neill et al. (2008) used a reciprocal transplant experiment to determine growth across the fundamental niche of lodgepole pine (*Pinus contorta*). The authors’ results suggest lodgepole pine should be planted more in many northern coastal areas since the species may grow well under projected climate warming in these regions provided there are no moisture limitations. However over the past decade epidemic levels of dothistroma needle blight, caused by the increased summer precipitation for the region (Woods et al. 2005). Thus, if transfer decisions solely follow O’Neill et al.’s projections, precipitation increases anticipated for this region could result in epidemic disease outbreaks, negating the expected productivity gains.

As was presented for cedar leaf blight (*Didymascella thujina* (E.J. Durand) Marie) and western red cedar (*Thuja plicata* Donn ex D. Don) in British Columbia, projections of disease risk can be useful for refining assisted migration strategies when adequate disease observations accompanied by information on the natural genetic variation in disease resistance among tree

species populations is available. However, there are a number of factors that need to be addressed when projecting the risk of forest disease under uncertain future climates.

First, incidents of forest disease are often largely driven by cumulative climate events that occur over extended periods of time. Consequently the climate that instigated disease occurrences is not necessarily reflected in the year that the disease is measured. For example, the cedar leaf blight disease cycle occurs over two growing seasons with infection occurring in the first year, and disease expression and spread appearing the year following (Trotter et al. 1994). Ascospores are produced from July to October, peaking between late August and early November (Sinclair et al. 1987), however germination commonly starts in the spring by ascospores that have overwintered on green foliage from the previous year (Frankel 1990). Each one of these events requires a specific range of temperature and moisture regime to occur which may not be accurately captured by standard average annual, seasonal or monthly climate variables for a given measurement year. Second, low incidents of infection can be difficult to observe which can cause key climatic conditions associated with a disease outbreak to be overlooked.

Third, gaps in our understanding of which climate factors most influence disease outbreaks could bias future disease projections. For example cedar leaf blight is present at very low levels in the interior of British Columbia as well as at high elevation environments which characteristically have colder winter temperatures. This pathogen has the ability to overwinter below -5°C and continue its growth cycle the following year (Kope 2004), but little is known about the pathogen’s minimum temperature threshold. A greater understanding of the influence extreme minimum temperatures is having on pathogen survival in these environments could influence future projections of disease risk given warming trends are expected to be greatest in the winter months (Mbogga et al. 2009).

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Lastly, relative humidity is often considered the most important climatic factor for influencing sporulation, germination and fungal growth of forest pathogen species; however it is also one of most difficult climate variables to accurately measure. While localized data loggers would be needed to properly capture humidity information, climate moisture indices that measure the difference in cumulative precipitation and evapotranspiration for a given region, offer an alternative that can be scaled up to assess species-wide risk.

Predicting how climate change will affect pathogen-host interactions in forest ecosystems is complex given increases in temperature and precipitation could favor the tree species, the pathogen or both. For example, pathogens will likely have the ability to adapt to new climates faster than their long-lived hosts (Sturrock et al. 2011). Considering the uncertainty in pathogen-host interactions as well as in what climate shifts will emerge in the future, it is important that assisted migration strategies be resilient to the negative consequences anticipated from forest pathogens. Comparing disease projections under multiple future climate change scenarios provides a range of potential forest health outcomes which allow managers to make more informed decisions. However to be resilient to negative consequences, managers must consider the likelihood that climate conditions associated with an epidemic disease outbreak will materialize. If the likelihood of this climate occurring is high, to be conservative assisted migration strategies must plan for this scenario with alternative management strategies including deploying resistant populations.

Projections of the future risk of cedar leaf blight on western red cedar in British Columbia under multiple climate change scenarios can be found as a peer review article submitted to *Agricultural and Forest Meteorology* November 2012.

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PANEL: TROPIC FOREST TREE DISEASES





OPERATIONAL DISEASE SCREENING PROGRAM FOR RESISTANCE TO WILT IN *ACACIA KOA* IN HAWAII

Nick Dudley¹, Robert James², Richard Sniezko³, Phil Cannon⁴, Aileen Yeh¹, Tyler Jones¹, and Michael Kaufmann¹

INTRODUCTION

In Hawaii, koa (*Acacia koa*) is a valuable tree species economically, ecologically, and culturally (Baker et al. 2009). With significant land use change and declines in sugarcane, pineapple, and cattle production, there is an opportunity and keen interest in utilizing native koa in reforestation and restoration efforts (Newell and Buck 1996; Elevitch et al. 2006). However, moderate to high mortality rates in many low to moderate elevation plantings have impeded past efforts (Figure 1).



Figure 1. *Acacia koa* trees killed by *Fusarium oxysporum* (FOXY) at Kokee, Kauai.

The primary cause for this mortality, particularly in young plantings, is koa wilt disease, caused by *Fusarium oxysporum* f. sp. *koae* (FOXY) (Gardner 1980; Sun 1996; Daehler et al. 2002; Shi 2003). *F. oxysporum* is a relatively common agricultural and nursery fungus, but the origin of strains of FOXY virulent to koa in Hawaii is unknown. *Fusarium oxysporum* is primarily a soil-dwelling fungus,

which typically invades susceptible plants through the root system (MacHardy and Beckmann, 1981). Upon entering the roots, successful infection requires entrance into the plant's xylem tissue, where it is then able to spread upwards, leading to the disruption of water movement and eventual plant death (MacHardy and Beckmann 1981). The exact mechanism the pathogen utilizes to disrupt water flow varies by host and is dependent on specific host-pathogen interactions. The nature of these interactions between koa and FOXY is unknown. Identifying and developing koa populations that are genetically-resistant to virulent strains of FOXY may be the key to successful koa restoration and reforestation (Sniezko 2006). Great differences in mortality among seed sources in young koa field trials planted in the 1990's was the impetus for developing a seedling screening test and investigating genetic resistance to FOXY (Sniezko 2003).

ISOLATE COLLECTION

A state-wide survey was conducted to determine distribution of koa wilt/dieback disease across the four main Hawaiian Islands: Kauai, Maui, Oahu and Hawaii (Figure 2). A total of 386 samples were taken at 46 different sites covering approximately 13,830 acres of natural and planted koa forest (James et al. 2007a; James et al. 2007b). Koa trees and seedlings infected by FOXY were found on all of the major islands in forest tree seedling nurseries, natural, and plantation forests. From these samples more than 500 isolates of FOXY were obtained. All the isolates were identified using morphological characterization (Nelson et al. 1983; James 2007a).

ISOLATE SCREENING

A greenhouse based pathogenicity test was developed to distinguish between nonpathogenic and pathogenic strains and to identify highly virulent isolates for use in resistance screening (Dudley et al. 2007; Dudley et al. 2009).

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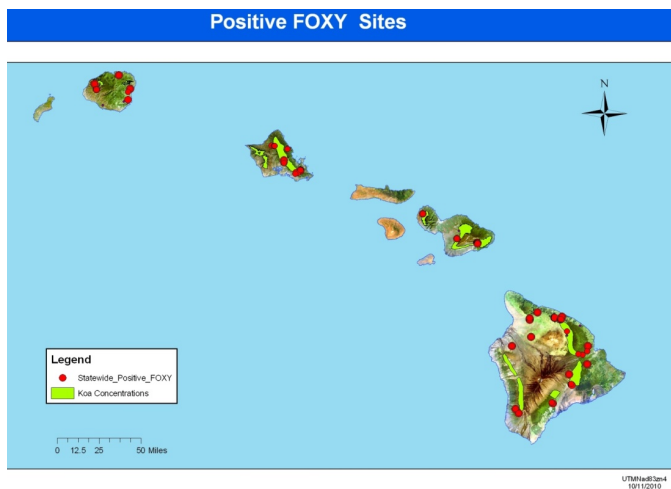


Figure 2. Principle location of koa stands in Hawaii and sites where koa root samples have been positive for FOXY.

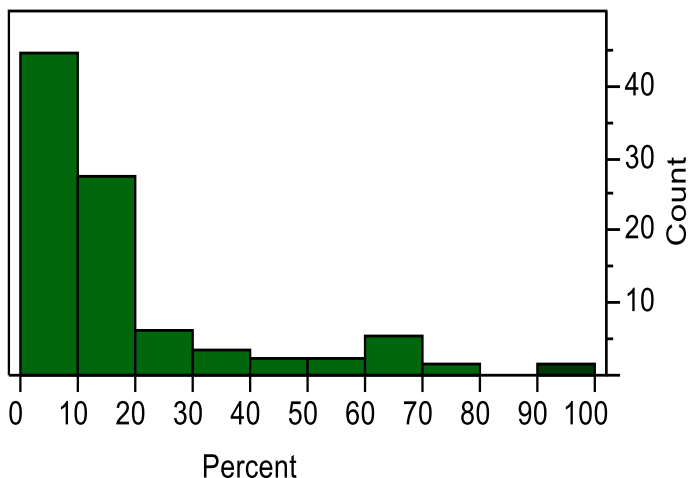


Figure 3. Numbers of FOXY isolates that cause different levels of koa seedling mortality.

One-hundred and sixty of the collected FOXY isolates were screened for virulence on koa seedlings in greenhouse inoculation trials. The isolates were grown on a cornmeal, potato dextrose agar, and perlite substrate and then incorporated into soilless potting mix. Twenty-five koa seedlings were transplanted directly into the inoculated potting mix for each isolate. The seedlings were monitored for 90 days. Seedling mortality was assessed biweekly, at which point wilted seedlings were removed from their growing containers, surfaced sterilized, and sent for reisolation. FOXY was reisolated from over 99 percent of the dead seedlings.

The mortality rates varied widely, and served as the basis for assigning virulence. The majority of isolates

were non-pathogenic, or had low virulence (Figure 3). Ten highly virulent isolates were selected for use in greenhouse resistance screening trials (Figure 4).

RESISTANCE SCREENING

Between 2006 and 2011, more than 350 koa families were evaluated for their potential FOXY resistance in greenhouse tests (Figure 5). Single tree collections were made from open pollinated trees, resulting in half-sib families. Most of the seed was collected from wild populations but collections were also made from survivors of family level progeny trials at the HARC's Maunawili Field Station, Oahu, Hawaii (Figure 6). A composite of ten virulent isolates of FOXY were used for inoculation (Dudley et al. 2009). Seedling inoculation was similar to the isolate trials, except the virulent isolates were mixed together prior to being incorporated into the potting mix. Twenty-five to thirty seedlings were inoculated per family, and the trial was organized as a randomized row plot design, with 5-8 replicates per trial. Seedling wilting and mortality in the greenhouse was monitored over a 90 day period for each test. Mortality typically began around day 20, peaked at day 40 and approached zero by day 90. Wilted seedlings were recorded, surface sterilized and sent for reisolation. A subset of the surviving seedlings was also sent for reisolation. FOXY was reisolated from over 95 percent of the wilted and surviving seedlings.

Host	Host Information	Percent
Hawaii	Fine tree roots;	72 ¹ , 60 ³
Hawaii	Fine tree roots;	44 ² , 40 ³
Kauai	Fine tree roots;	60 ¹ , 44 ³
Kauai	Rhizosphere;	80 ¹ , 52 ³
Kauai	Stem; outer; wilted	88 ² , 92 ³
Kauai	Stem; inner; wilted	68 ¹ , 72 ³
Oahu	Seeds/young	44 ¹
Oahu	Seeds/young	68 ¹ , 56 ³
Oahu	Seeds/young	56 ²
Maui	Roots; wilted tree	60 ³

Figure 4. The ten most damaging FOXY isolates and the amount of mortality that they caused in one or more of three seedling inoculation trials. The superscripts in the mortality column indicate the number of the inoculation trial.



Figure 5. Typical layout of an experiment to evaluate about thirty different koa half-sib families to a composite of the ten most pathogenic isolates of FOXY. The soil mix contains the inoculum; the trial is underway at the Hawaiian Agricultural Research Center (HARC) at Maunawili.



Figure 6. A fourteen-year-old koa seedling seed orchard at HARC, Maunawili.

SUMMARY

The trials have proven effective in distinguishing resistance frequency by family. The overall survival average is approximately 40 percent, but varies greatly by family. The top families have over 75 percent survival at day 90, while the most susceptible families have 0 percent survival. The seed collected from trees at the HARC Maunawili Station had an increased frequency of resistance compared to that collected from native populations (Figure 7 and 8).

The Maunawili Station is at relatively low elevation (150m), and the site is known to have strong disease pressure. The top families from the trials were selected and planted in field trials in 2012 to evaluate the resistance over time. If the field trials demonstrate long term resistance, the trials will be converted into seed orchards to produce wilt resistant seed for future reforestation and restoration projects. Continued screening of additional koa families for pathogen resistance, retesting putative resistant families, and developing koa seed orchards with disease-resistant stock are either on-going or planned.

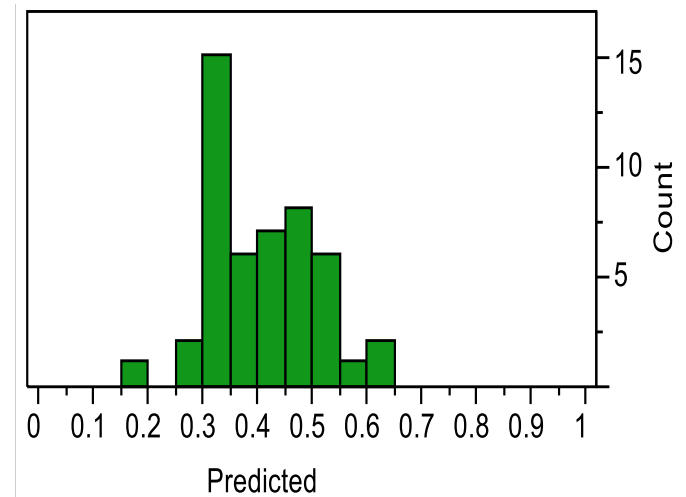


Figure 7. Numbers of Hawaiian half-sib families of koa with different survival rates out of the 160 families tested to date for FOXY resistance at HARC, Maunawili.

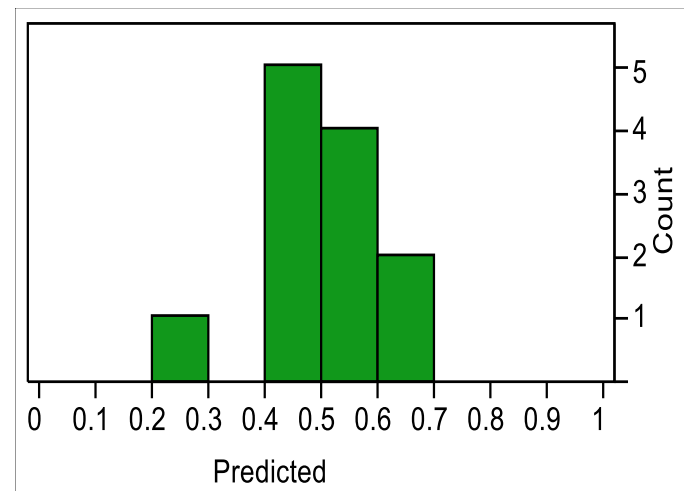


Figure 8. Numbers of half-sib families of koa with different survival rates out of 12 families from the Maunawili seed orchard tested for FOXY resistance.

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INTEGRATED PERSPECTIVE ON GUAM IRONWOOD TREE DECLINE

Robert L. Schlub¹, Karl A. Schlub², A. M. Alvarez³, M. Catherine Aime⁴, Phil G. Cannon⁵,
and Anand Persad⁶

ABSTRACT

Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in decline. Research on the cause of ironwood tree decline (IWTD) began in earnest in January 2009 when six invited off-island scientists, together with participants from Guam, took part in a 5-day IWTD conference (Mersha et al. 2009). The findings of that conference and subsequent research were published in the proceedings of the 4th International *Casuarina* Workshop, Haikou, China, in 2010 (Schlub et al. 2011). Therein, it was reported that a complex of biotic and abiotic factors were responsible for the decline. The theory that opportunistic conk-producing fungi such as species of *Ganoderma* and *Phellinus* as an explanation for the majority of Guam's declining trees was advanced. Much of what was identified as contributing IWTD factors has now been confirmed and will be clarified in this WIFDWC Proceedings. A conk-producing species in the *Ganoderma australe* complex was identified as the primary wood-rotter. This fungus was commonly found on Guam where IWTD is widespread and rarely on Saipan, a nearby island where the majority of trees are considered healthy. Bacterial colonization of the xylem is seen in trees with thinning foliage, which is indistinguishable from those attributed to ironwood decline. Two bacteria were consistently isolated: *Ralstonia solanacearum* and an unknown. We believe the unknown bacterium is responsible for the wetwood symptom associated with Guam's declining trees and that both bacteria play a role in Guam's ironwood decline. With the addition of GIS map derived variables to the original model, it was found that trees are less impacted by ironwood decline when there is adequate soil moisture holding capacity, when trees are in a forest setting or in a properly managed landscape such as a golf course. Likewise, the amount of declining trees at a given site can be expected to intensify with increases in the occurrence of conks and termites, height above sea level and tree size. The current model will be strengthened, with the addition of *R. solanacearum* survey data. When tree circumference and dieback maps were compared, tree site productivity could not explain the high level of IWTD predicted in central Guam. The increased presence of termites, conks and storm damage with increasing tree size suggests that under ideal tree stand conditions these variables are part of the normal process of tree senescence.

INTRODUCTION

History

Casuarina equisetifolia, which is native to Guam and locally known in the English language as ironwood and in the Chamorro language as “gago,” has been continually propagated on Guam since the 1600s. As a result of its nitrogen-fixing ability and tolerance to salt spray and typhoon damage, the tree is able to thrive in the Mariana Islands where typhoons and coral sand beaches and other nutrient-poor soils are commonplace.

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Botanical Characteristics

The tree is an evergreen and its needle-like jointed branchlets bear the anatomical minute tooth shaped leaves. As a result of limited leaves and floral structures, the tree has the ability to conserve moisture and tolerate drought. Within the Mariana Islands the average lifespan of ironwood is estimated to be 35 to 90 years with an average maximum height and circumference at breast height of 13.7 and 2.9 m, respectively. Due to damage from typhoons in the Mariana Islands, exposed trees are often topped with prolific epicormic shoots, which results in a shorter tree with a wider crown than what is typically seen in Hawaii.

Ecology

Ironwood thickets are a component of Guam's forestland, where it is considered a secondary forest species (Liu and Fischer, 2006). Ironwood trees do not

compete with native tree species in undisturbed limestone forests (Moore, 1973), although it grows nearly everywhere: beaches, landfills, road shoulders, cleared land, and vacant lots. In the Mariana Islands it grows both in savanna grasslands in clay volcanic soils and in coastal strands in calcareous sands and loamy sand soils. In large dense strands trees produce a thick, slowly decomposed, allelopathic litter layer that eliminates nearly all understory vegetation.

Several prominent forest features of ironwood on Guam were mentioned in a 2002 Guam Forest Bulletin (Donnegan et al. 2004). Ironwood trees were reported to be among the healthiest trees on island with an estimated population of 115,924 for trees greater than 5 in. in diameter at breast height. *C. equisetifolia* was mentioned as a prominent member of the halophytic (sea-salt adapted) vegetation type. This vegetation is found along beaches in the north and south, where it may be composed solely of ironwood or a mixture of other species including *Cocos nucifera*, *Guettarda speciosa*, *Hernandia sonora*, *Pandanus tectorius*, *Scaevola taccada*, *Thespesia populnea*, and *Tournefortia argentea*.

Pests and Diseases

Guam's ironwood tree insects and pathogens are generally considered incidental or opportunistic. Damage by incidental pests are precluded primarily by abiotic disorders. Drought periods especially during the dry season will primarily affect plants in poor planting sites where the trees become stressed and consequently become vulnerable to these insects and pathogens. Some pathogens may be agents of latent infections; therefore, the infection precedes environmental changes that trigger symptom production.

In India, termites feed on underground roots and stems of live *Casuarina equisetifolia*. This type of damage is believed to be occurring in Guam as well. From past entomological surveys and reports, colonies of *Nasutitermes* sp. and *Microtermes* sp. were found feeding on dead ironwood trees (Moore, A. personal communication). The Philippine milk termite *Coptotermes gestroi* was responsible for killing ironwood trees transplanted onto a new golf course (Yudin, L.S., personal communication).

Damage to branchlet tips by an unidentified gall wasp is known to reduce branchlet length and total branchlet mass (Mersha et al. 2009). The impact on tree health is probably negligible but may be significant on trees with thinning foliage. The wasp reared from branchlet tip galls was identified as belonging to the genus *Selitrichodes* (*Eulophidae: Tetrastichinae*) by John LaSalle, CSIRO, Australia.

Ralstonia solanacearum the cause of bacterial wilt is among the most commonly worldwide reported pathogens of *Casuarina*. It is a xylem-resident bacterium mainly entering via roots. Though only occasionally reported as serious, bacterial wilt has emerged as the most serious disease of *Casuarina* in China (Huang et al. 2011) after its discovery in 1964. Based on field observations, culturing from symptomatic tissues, immunostrip data, LAMP data, and other tests, it was concluded that *R. solanacearum* is part of the disease complex described as ironwood decline in Guam (IWTG).



Figure 1. Bacterial wilt of *C. equisetifolia* sapling in China (photo provided by Dr. Chonglu Zhong).

In addition, an unidentified companion bacterium hereby referred to as Guam strain was associated with the wetwood symptoms also associated with declining trees. Thus, two xylem-resident bacterial species are associated with IWTD. The Guam strain (rod shaped and motile after 24 hours) is faster growing and more abundant than *R. solanacearum*. On Guam, trees that harbor these two bacteria do not manifest the same symptoms as those observed in China.

In China, the field symptom is rapid tree death (Figure 1), which is triggered by severe environmental stress such as a typhoon. On Guam, bacterial colonization of the xylem results in trees with thinning foliage, which is indistinguishable from symptoms associated with IWTD (Figure 2). Differences between China and Guam diseases can also be seen in symptoms revealed in cross-sections of the trunks and limbs. In China, xylem vessels of trunk cross-sections contain diffused areas of slightly darker tissue and yield copious amounts of bacterial ooze (Figure 3). On Guam, cross-sections of infected trees revealed uncontained areas of dark discoloration "wetwood", with sharply defined borders that radiated from the center of the tree. Droplets of bacterial ooze may or may not appear and are generally restricted to the "wetwood" which has a high moisture content (Figure 4).



Figure 2. Comparison of health *C. equisetifolia* tree, DS=0, (left) with first level of Decline Severity, DS=1, (right). Note thinning foliage and slight tip-dieback of fine branches.

Ooze consistently contained both the Guam strain and *R. solanacearum*. Both bacteria are translocated through xylem vessels of *C. equisetifolia* seedlings following wound inoculation with the bacteria that oozed from infected wood. We believe the Guam strain is

responsible for the wetwood symptom associated with Guam's declining trees and that the Guam strain itself plays a role in Guam's ironwood decline. There are many fungi involved in wood rot or decay, one group are the basidiomycetes. The sporocarps of the wood rotters found in Guam and Saipan were either flat (resupinate) or shelflike (conk) (Figures 5 & 6). To date, five conk-forming basidiomycete genera have been identified from ironwood on Guam, all in the class Agaricomycetes: *Ganoderma*, *Favolus*, *Pycnoporus*, *Phellinus*, and *Sarcodon* (Schlub et al. 2011). The death of large branches and later roots may occur without the presence of sporocarps.

As a result of a survey in February 2012, the basidiocarp found to be the most frequently associated with unhealthy trees and wood-rot was that of (*G. australe* complex) (Figure 5) (Schlub et al. 2012). It invades woody tissue through an unrestricted mycelial network while sustaining themselves on cell and cell wall components (Figure 7). Its conks of appeared on roots and butts of declining trees and stumps of dead trees. On Saipan where decline does not exist and where the trees are considerably healthier, *G. australe* complex was rarely found, and then only in association with a few unhealthy appearing trees.



Figure 3. Cross-section of a tree in China with bacterial wilt reveals copious amounts of bacterial ooze and tissue discoloration. From a presentation of Huang Jinshui, He Xueyou, Ke Yuzhu, Cai Shouping, Chen Duanqin, and Tang Chensheng of Fujian Academy of Forestry Sciences at International Casuarina Workshop Haikou, China 21-25 March 2010.



Figure 4. Cross-sections of infected *C. equisetifolia* tree revealed expanding areas of moist discolored wood (wetwood) that radiated from the center of the tree accompanied by droplets of bacterial ooze composed of *Ralstonia solanacearum* and an unknown bacterium.



Figure 5. Sporocarp (conk) of *Ganoderma australe* species complex on *C. equisetifolia*. On Guam, 100 percent of the trees in decline sites may have conks on their roots or butts.

IRONWOOD TREE DECLINE

Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in the midst of a decline that was widespread by 2005. Several trees stands on Guam began showing decline symptoms in 2002 and 2003 following typhoons Chata'an in July and Pongsona in December of 2002. Observations by Bart Lawrence, long time resident and forester, believes decline may

have begun in isolated areas following typhoon Gay in November 1992.

Ironwood Tree Decline (IWTD) starts with thinning of foliage and slight dieback of fine branches at DS=1 (Figure 3) and processes to the final stage (DS=4) characterized by dieback of large branches and 95 percent defoliation. At DS=1, the outward symptom of IWTD is indistinguishable from those produced by Guam's two xylem-resident bacteria. Internal symptoms (as seen in trunk cross-sections) vary from tree to tree and with decline severity. Small trees (< 50 CBH) and those at DS=1 generally have symptoms associated with bacterial infection of the xylem, others have no bacterial ooze and only a small area of centrally-located, contained discoloration. Medium size trees and those at DS=2 usually have bacterial symptoms (Figure 4), and less common signs of wood rots caused by *Ganoderma* (Figure 7) and termites. Trees in a severe state of decline harbor one or all of the following: bacteria, termites, various resupinate sporocarps and conks of *Ganoderma australe* species complex (Figure 5), *Phellinus* (Figure 6) and other Agaricomycetes.

Statistical Modeling

Modeling was used to evaluate a set of data from 1427 individual trees, 44 sites, and 16 GIS maps.

Analysis of Individual Trees

For each sample tree, a set of measurements were taken and selected for analysis (Table 1). The primary objective of using statistical models with the ironwood tree data is to find possible factors that could be related to tree decline, in other words to find the parameters that have a positive or negative impact on the tree (Schlub, 2010). Various modeling techniques were applied to address data set issues. The logic model, which used dieback as the response variable, was found to be the best fit with the data.

Three explanatory variables were found to be significant and therefore could explain the ironwood's state of health (Table 1). Among the three regressors, presence of conks had the largest coefficient value at 3.31. The impact of each individual regressor was determined numerically by holding all other regressors constant. When applied to conks, the odds of a tree being in decline would be 27.3 times in comparison to a tree that did not have a conk.

Table 1. Grouping and descriptions of ironwood tree variables, those in bold were found to be the most suitable for predictive purposes.

Response Variables				
Decline severity	Severity ranking DS=0,1,2,3,4			
Tree Dieback	Healthy or unhealthy			
Explanatory Variables				
Structure	Number of trunks per tree	Circumference of tree at 1.3 m height	Site density: trees per square meter at site	
Stress	Fire damage: present or not	Conks: present or not	Storm damage: present or not	Termites: present or not
Geographic	Latitude	Longitude	Altitude	Site
Miscellaneous	Level of lawn management: none, moderate, high	Tree origin: natural or planted		



Figure 6. Sporocarp (conk) of a *Phellinus* sp. on *C. equisetifolia* trees. This fungus is likely a part of the normal decay process of the ironwood trees in the Marina islands and not a contributor to IWTD or stand losses.

Analysis of Tree Sites

Tree sites were examined using the original tree explanatory variables (Table 1) plus those derived from 16 GIS map characteristics (Kennaway, 2010): cemetery buffer, FIA trees with conks (multi-ring buffer); fire risk; fires per year; proximity to golf courses; land cover; management areas; school buffer, soil available water at 150 cm, available water at 25 cm soil depth; soil depth to restrictive layer; soil series; and vegetation. Some maps were dropped from the analysis

because of correlations between regressors. A multiplicative change in the odds ratio of unhealthy vs healthy was calculated one regressor at a time by increasing the regressor one unit and holding all remaining regressors constant.

There were 6 positive IWTD predictors: increases in circumference, increases in altitude, presence of conks, presence of termites, planted stand vs natural stand, and urban land location.

There were 4 negative IWTD predictors: increasing water availability at 25 cm soil depth, golf course location, forest location, and decreases in latitude.

In summary, the most beneficial variable identified was soil moisture. Trees in areas with the highest moisture were 3.3 times less likely to be declined. Likewise, the most deleterious variable was the presence of conks. Trees with conks were 27 times more likely to be in a declined state.

Predicting Tree Size

As a result of multi-linear modeling, several factors were identified that may positively (+) or negatively (-) predict the average size of trees at a site (cm in circumference at 1.3 m). The size of a tree is restricted by tree stand density, altitude, and soil depth.

Sites with large trees are more likely to be found in urban, forest, national parks, and fire prone areas, than in sites at golf courses or in close proximity to a school. It was also found that increased circumference is associated with trees having termites, conks, typhoon damage, and multiple trunks. This suggests that large highly vigorous trees are able to tolerate stresses to which less vigorous trees would have succumbed.



Figure 7. Cross-section of rotted ironwood tree butt infected with *Ganoderma australe* species complex. Note expanding network of white mycelial strands.

Linking Dieback with Site Productivity

Based on the premise that tree circumference in 2008 and 2009 is an indicator of site productivity, an association between IWTD and circumference was sought. The circumference map supports the concept that nearly the entire island is suitable for the growth of small trees (Figure 8). However, as the size of the trees increases the area suitable for sustained growth decreases. When the map for dieback (Figure 9) was visually compared to the map for circumference, IWTD appears poorly linked to site productivity (circumference) and strongly linked to the central area of Guam. This suggests that IWTD is not a natural progression of tree maturation and death. Many factors have been evaluated as possible causes or contributors to ironwood decline, those that have some perceived relevance by the authors are listed in Table 2.

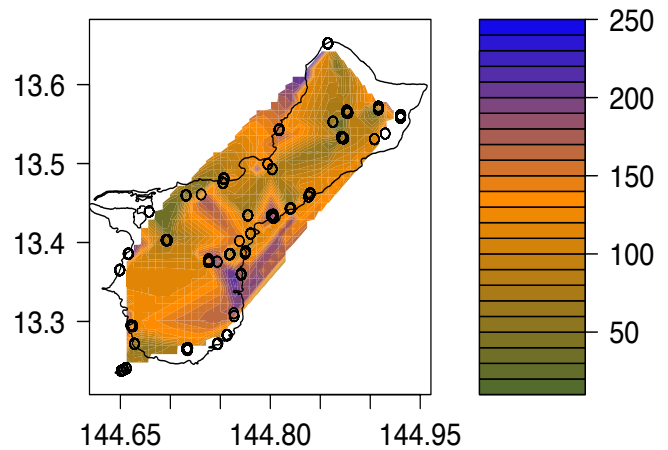


Figure 8. Map of tree circumference over a longitude-latitude grid of the island of Guam; hence, areas of large trees sites (purple color) have habitats more suitable for ironwood growth irrespective of the presence of IWTD.

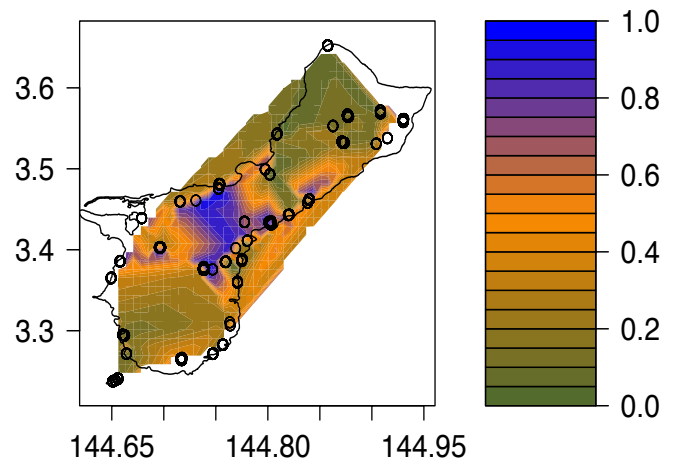


Figure 9. Map of the predicted probability of dieback using a logistic model. Areas in blue indicate regions where dieback is most likely to occur.

RECOMMENDATIONS

Due to the slow progression of IWTD and its general sporadic nature, decline on Guam could be reduced substantially through cultivar selection and cultural practices which promote healthy growth and preclude favorable conditions for pests (termites) and pathogens (wood-rots, root-rots and bacteria). Currently, experiments are underway to identify new provenances of *C. equisetifolia* that might grow well under pressure from Guam's pests, diseases and typhoons. There are 23 provenances in this test which represent *C. equisetifolia* sources from around the globe.

Table 2. Likely contributors to ironwood decline and their perceived relevance from low * to high ****.

Biotic factors	Emerging factors	Relevance
Branch dieback	<i>Pestalotiopsis</i>	*
Root rot	<i>Fusarium</i> ,	*
Wood rot	<i>Ganoderma</i>	****
Xylem residing bacteria	<i>Ralstonia solanacearum</i>	***
	wetwood associated	**
Nematodes	<i>Helicotylenchus</i>	*
Insects	Termites	**
	Gall wasp	*
Abiotic factors		
Weather	Typhoon damage	*
Management	Poor tree care practices	*
Site environment	Poor site selection	**
Host genetics	Lack of genetic diversity	**

Experimental plans are also underway to determine if there are particular spacing patterns or pruning practices that can enable ironwood tree to be more wind resistant. Good site selection and good establishment techniques are among the recommended practices for avoiding ironwood decline along with all activities that could lead to the damage of the cambium in the boles of the stem. Grass trimming near trees and ground fires are estimated to have been particularly detrimental to ironwood trees in the past couple of decades.

Site Evaluation and Soil Attributes

Site evaluation and soil care before planting ensures a healthy transplanted plant with increased tolerance to transplant shock as well as a tree that will reach its full maturity. Ironwood is suited for a range of sites and locations. Its growth habit dictates that it be planted 40 ft. from houses and 20 ft. from each other. In urban, windrow, and agro-forestry situations closer spacing may be necessary.

The island of Guam has three broad landform categories each with their own set of soil parent materials, which are responsible for the formation of 8 major soil units each with unique chemical and physical attributes (Figure 10). Chemical attributes of a soil are those related to the activity of ions within the soil solution; measurements include pH and CEC. Though ironwood can grow across Guam's wide range of soil pH, soil nutrients are maximized between pH 6-7. Cation Exchange Capacity (CEC) is a measure of the soil's

ability to hold onto nutrients, which increases with a soil's fertility.

The physical attributes of a soil are those related to the size and arrangement of its solid particles. Measures of physical properties include soil bulk density; soil texture, soil porosity or percolation Bulk density is an indicator of soil compaction, which is an indicator of root growth and soil porosity or percolation. The majority of the island of Guam has clay soils with bulk densities of 0.60-1.0 g/cm³, which are ideal for clayey soil. Unfortunately the soil is often no deeper than 16 cm. The permeability or percolation rate for Guam's soils vary widely from poor (0.1 in. or less / hour) to rapid (5.0 in. or more). Poor soils should be avoided or modified as they promote shallow rooting, poor growth and root rots. Rapid soils are fine for ironwood, provided their roots can reached the water table, which will be critical for their survival in the dry season. Soil in an ideal state for tree growth contains 50 percent solids (45 percent mineral material and 5 percent organic matter) and 25 percent each of air and water.

Site Remediation

Compacted soil in or near a planting pit should be remediated as necessary. The detrimental effects of planting in compacted soil may include decreased water holding capacity, poor infiltration rates and decreased aeration. These factors could contribute to decreased root penetrability and thus increased susceptibility to drought and transplant shock. Remediation methods include aeration and incorporation of organic matter to

improve porosity. Aeration is normally conducted using an air-tool or air-spade. Because Guam's productive layer is thin, vertical mulching also may add benefit to new planting sites. Vertical mulching, a process of drilling or blasting with an air-tool vertical holes in the soil into which conditioned porous soil is added.

Tree Installation

Plants should be installed in saucer-shaped hole / pit that allow for expansion of the root zone with minimal substrate resistance (Figure 11). Soil should be removed with as little disturbance of the soil's profile as possible. Due to Guam's poor subsoil, mixing of topsoil and subsoil should be avoided. When backfilled, the site's profile should match the original. To enrich the topsoil, amended with organic material. Large rocks on the side or bottom of the pit should be removed with a backhoe or cracked with an air-tool or auger. The hole should be free of rocks and debris. It is a misconception that adding rocks or gravel in the bottom of the planting hole improves drainage. Care should be taken to avoid planting in holes with steep sides or made with a corer that compresses the sidewalls because in this scenario the roots could encircle amongst themselves leading to girdling roots. Balled or container trees must be carefully placed in the hole without disturbing the root ball. After installation, the tree should be staked.

Planting Bare Root Plants

After planting bare rooted trees, gently tap the soil and backfilling with water to remove air pockets. Additional staking may be required of bare rooted trees. Bare root plantings, although limited to smaller ironwood plants, allow for earlier adaptation to the new site and faster transplant recovery. However, a drawback in using this technique is that initially roots and the planting pit must be kept sufficiently moist to prevent roots from drying out. It is estimated that in Guam during the dry season early care should be administered for at least three months and about one month in the wet / rainy season. Early care consists of providing tree transplants a stress free environment, which may include daily watering.

Nutrient Management

Guam's soils benefit from nutrient augmentation especially in sandy soil and areas where soil has been disturbed. The soils of northern Guam are calcareous. Trees in these soils will likely benefit from the addition of chelated iron throughout their life time. Fertilizer

needs to be used sparingly as the development of nitrogen fixing Frankia and beneficial mycorrhizal will be held back with over application. A low nitrogen, slow release fertilizer with micro nutrients is ideal. Alternatively, apply a small amount (50 to 100 g) of a low analysis complete fertilizer (10-10-10) at transplant.

Mulching

Mulching or placement of organic material around the base of a new plant can be one of the most beneficial cultural practices for young ironwood trees. A mulch is anything used to cover the soil's surface for the purpose of improving plant growth and development. To be suited for plant growth, mulch must allow the exchange of air between the soil and the atmosphere and allow water to infiltrate into the soil profile. The selected mulch (eg ironwood needles) should be placed between 1-2 in. deep. Benefits of mulching include: conservation of soil moisture; moderation of soil temperature; improvement of soil quality (organic mulches); suppression of weeds; enhancement of landscape appearance; reduced maintenance, and protection of plants from damage caused by maintenance equipment.

Fertilizing

Fertilizing (also see nutrient management), especially in the early stages of planting, helps root development and may improve drought tolerance thereby reducing transplant shock.

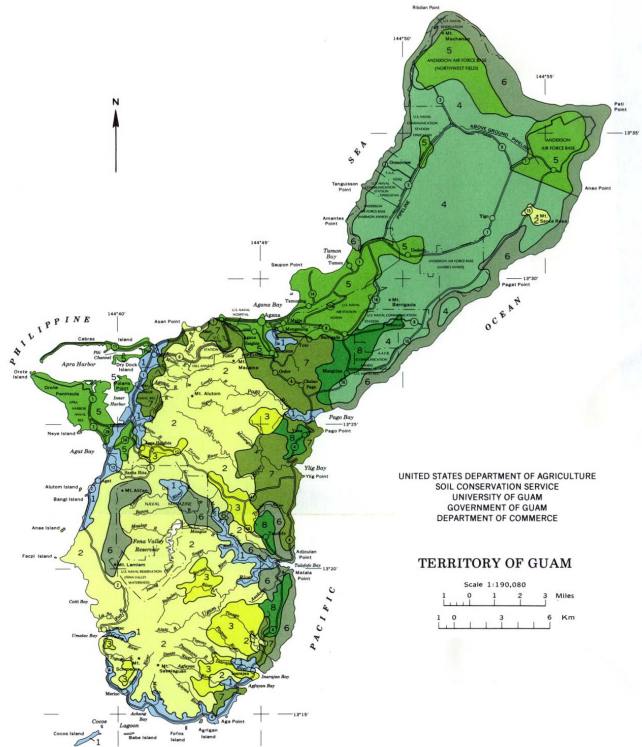
Watering

Watering or irrigation needs should be a part of the planning process, especially if planting is to occur in the dry season. Any irrigation program implemented should be based on knowledge of the soil percolation rates for the site. Excess moisture could lead to root rot.

Pruning

Pruning for health and training the young tree for structurally optimal strength relies on the judicious removal of plant tissue in a manner, as much as possible, consistent with minimal invasiveness to the plant. Proper pruning practices will enhance the overall health of the plant and should be guided by established standards. Tool sterilization is critical in ensuring sanitation and reducing the potential transfer of pathogens. Wind damaged trees should be correctly pruned as quickly as possible to reduce the amount of deadwood and reduce the surface areas of branches ripped in strong wind.

Removal of deadwood reduces the establishment of termites and wood-rotting fungi that contribute to hazardous trees in Guam's urban landscape. Trees broken from typhoons should be felled by excavation instead of sawing where their colonization by a wood rotting fungus could possibly lead to infecting the root systems of neighbouring healthy trees.



Key	Soil	Horizon depth (cm)	Clay (%)	Bulk density (g / cm ³)	pH	CEC
SOILS ON BOTTOM LANDS						
1.1	Inarajan-Inarajan	0-13	50-70	0.90-1.10	5.1-7.3	51
1.2	Shioya	0-25	0-3	1.10-1.25	7.4-8.4	7
SOILS ON VOLCANIC UPLANDS						
2	Akina-Agfayan	0-10	45-80	0.80-0.95	5.1-7.3	23
3	Akina-Togcha-Ylig	0-13	45-70	0.85-1.10	5.1-6.5	36
SOILS ON LIMESTONE UPLANDS						
4	Guam	0-25	35-55	0.60-0.90	6.6-7.8	22
5	Guam-Urban land-Pulantat	0-25	35-80	0.60-1.10	6.6-7.8	27
6	Ritidian-Rock outcrop	0-10	35-40	0.70-0.90	6.6-7.8	—
7	Pulantat	0-16	70-90	0.90-1.10	6.1-7.3	31
8	Pulantat-Kagman-Chacha	0-20	40-80	0.90-1.20	6.1-7.8	26

Figure 10. General Soil Map of Guam (Young et al.1988).

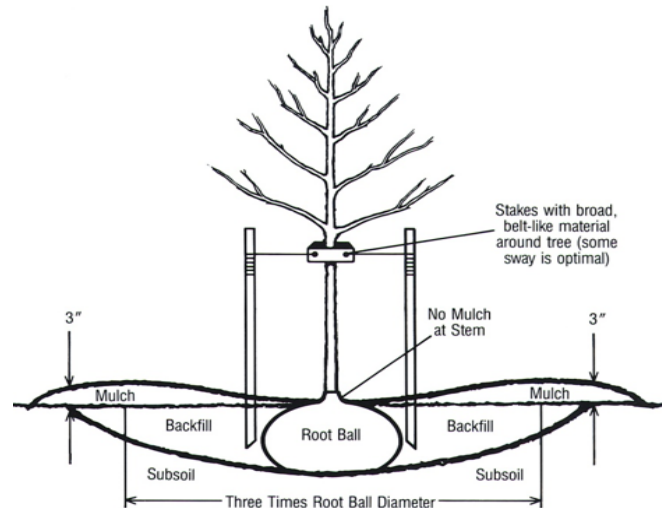


Figure 11. General installation/hardware guidelines for tree installation.

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INFECTION RATES OF FIVE BRAZILIAN STRAINS OF *PUCCINIA PSIDII* ON SIX FAMILIES OF HAWAIIAN OHIA (*METROSIDEROS POLYMORPHA*)

André Costa da Silva¹, Pedro Magno Teixeira de Andrade¹, Acelino Couto Alfenas¹, Rodrigo Neves Graça², Phil Cannon³, Rob Hauff⁴, Diego Cristiano Ferreira¹, and Sylvia Mori⁵

ABSTRACT

One strain of *Puccinia psidii*, the causal agent of rust of Myrtaceae, was recently reported on multiple myrtaceous hosts in Hawai'i, but it has caused only very mild levels of damage to the state's predominant native forest tree, ohia (*Metrosideros polymorpha*). Earlier reports on this disease to WIFDWC have shown that there are multiple other strains of *P. psidii* in Brazil which are not present in Hawai'i and these were identified and characterized by microsatellite analyses.

This report covers a split-plot experiment that was conducted in Viçosa, Brazil to determine the pathological impact of five of these Brazilian strains of *P. psidii* on ohia and to assess variation in susceptibility of six different ohia families to each rust strain. It also covers another experiment that was run to determine the influence of the rust on growth and survival of ohia seedlings.

Three of the five *P. psidii* strains were highly pathogenic to most inoculated ohia seedlings; the other two were much less virulent. None of the ohia families used in this test showed significant resistance to the three most pathogenic strains, but some ohia families were especially susceptible to specific strains. The infection by *P. psidii* reduced height growth of ohia seedlings by 69 percent and increased mortality by 27 percent at 6 months post-infection, compared to uninfected controls.

INTRODUCTION

In 2005, the rust pathogen *Puccinia psidii* was first reported on an ohia seedling (*Metrosideros polymorpha*) growing in a greenhouse in the Hawai'ian Island of Oahu (Killgore and Heu 2007). Subsequently, this rust was reported on all major islands of Hawai'i (Uchida et al. 2006; Anderson 2012). By April of 2012, this rust had been found to infect young, unsclerified, foliage of 31 myrtaceous tree and shrub species (Janice Uchida, pers. com.*) out of the approximately 200 myrtaceous species occurring in Hawai'i.

In Hawai'i, *P. psidii* infection has had variable impacts among myrtaceous species. The most striking impact has been on rose apple (*Syzygium jambos*), an invasive plant that had previously colonized tens of thousands of

hectares in Hawai'i. This plant has experienced heavy defoliation wide-spread mortality following infection. *P. psidii* has also had a devastating impact on *Eugenia koolauensis*, one of Hawai'i's endangered endemic species. However, the impact of *P. psidii* infection on most of the other myrtaceous hosts, including ohia, has been less severe to date.

It is especially fortunate for Hawai'i that *P. psidii* has had only light impact on ohia, because the ohia tree is the most wide-spread tree species in Hawai'i; it constitutes approximately 80 percent of all forest trees in Hawai'i's rainforests (Loope and Uchida 2011) and occupies about 405,000 hectares (1,000,000 acres) in Hawai'i based on data provided for ohia and koa distribution by DOFAW (2002) and on koa distribution by Baker et al. (2009). For these reasons, ohia is the most critical species for protecting Hawai'i's watersheds and native ecosystems which provide habitat for much of the state's wildlife (Loope and Uchida 2011).

Although *P. psidii* has had only a light overall impact on Hawai'i's natural ohia so far, concerns remain about

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potential future impacts of this rust pathogen on the ecologically important ohia trees. Specifically, research by Zhong et al. (2008, 2011) indicated that only one genotype of *P. psidii* had been introduced to Hawai'i. Thus, scientists were concerned that other strains of this rust could be accidentally introduced to Hawai'i and that some of these strains might be more aggressive on ohia (Loope and Uchida 2011). Of further concern, the variation in rust susceptibility among ohia sources has not been well characterized, so it remains unknown which populations are more vulnerable to this rust.

To address both of these concerns, an extensive sample of *P. psidii* isolates was collected from diverse myrtaceous species across widely ranging geographic locations in Brazil. Subsequently, the genotypes of these *P. psidii* isolates were determined by microsatellite analysis. This analysis indicated that multiple *P. psidii* genotypes were present in Brazil with varying genetic relationships (Graça et al. 2011a, 2011b; Figure 1).

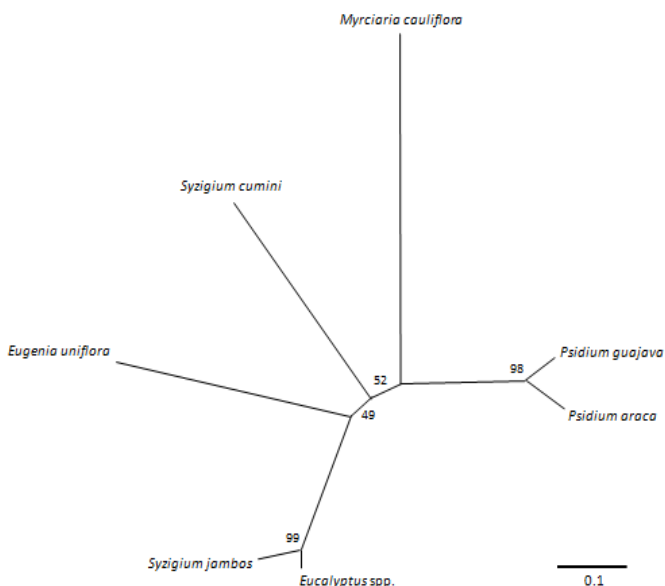


Figure 1. Neighbor-joining tree of the multilocus microsatellite genotypes showing the relationships among *Puccinia psidii* isolates from seven different hosts and locations in Brazil. Bootstrap values based on 1000 replications are shown. Branch length corresponds to genetic distance between genotype groups (From: Graça et al. 2011b).

Based on the results of this first study (Graça et al. 2011a), the aim of this present work was to assess variability in the potential pathogenicity of several of

these Brazilian strains of *P. psidii* on seedlings from a range of ohia families as well as to estimate the impact of severe *P. psidii* infections on growth and survival of ohia seedlings.

MATERIALS AND METHODS

Two studies were conducted at the Universidade Federal de Viçosa, Viçosa, Brazil:

Susceptibility of Ohia Families to Different Strains of Puccinia psidii

To test differences in susceptibility among ohia families, at least 300 ohia (*Metrosideros polymorpha*) seed were collected from six different open-pollinated (OP) ohia trees growing on the island of Hawai'i. These trees have the following codes and geographic points of origin:

- Family 1 080416001 002 UTM 0307613E 2154842N (var. *incana*) Kapoho 25 masl.
- Family 5 080416002 001 UTM 0265175E 2149255N (var. *glaberrima*) Volcano 1177 masl.
- Family 7 080416002 003 UTM 0259593E 2178405N (var. *glaberrima*) Saddle Rd. 1293 masl.
- Family 8 080416002 004 UTM 0259600E 2178383N (var. *glaberrima*) Saddle Rd. Elev 1284 masl.
- Family 11 080416003 003 UTM 0256929E 2177444N (var. *polymorpha*) Saddle Rd. 1452 masl.
- Family 12 080416003 002 UTM 0259749E 2178387N (var. *polymorpha*) Saddle Rd. 1232 masl.

These seed collections were processed in accordance with the requisite phytosanitary procedures and shipped to Brazil. The ohia seed were then germinated in the greenhouse of the Universidade Federal de Viçosa, and established as potted seedlings.

For tests on the aggressiveness of different *P. psidii* strains on ohia, the following five Brazilian strains of *P. psidii* were used:

- Strain 1 – derived from *Eucalyptus grandis* strain UFV2 (race 1), from Itapetinga, Sao Paulo State

(Jungmans et al. 2003b) S22.5986 W58.8003, 550 masl.

- Strain 2 – derived from *E. urophylla* x *E. grandis* hybrid strain EUBA-1 (race 4) from Teixeira de Freitas, Bahia State (Graça et al. 2011a) S18.4865 W39.9849, 78 masl.
- Strain 3 – derived from *Myrciaria cauliflora* (jaboticaba) from Minas Gerais State. S20.6554 W42.8367, 675 masl.
- Strain 4 – derived from *Psidium guajava* (guava) from Minas Gerais State. S20.4097 W43.0507, 587 masl.
- Strain 5 – derived from *P. araça* (Brazilian guava) from Bahia State. S15.5903 W39.2892, 120 masl.

To amplify urediniospore numbers for inoculation tests, single-uredinal isolates of each strain were separately inoculated as a spore suspension (2×10^4 urediniospores mL^{-1}) to leaves on host plants of the same species from which the strains were originally derived. After 20 days in the inoculation chamber, newly formed urediniospores were collected to provide the inoculum for this experiment. The urediniospores were stored at -80°C until required for assay.

1. A split-plot inoculation experiment was set up in which the five main plots in each block consisted of the five *Puccinia psidii* strains described above, and the split plots in each block consisted of each of the six different ohia families. Each split plot contained three seedlings for one of the six ohia families. Three completely randomized replications (blocks) of this design were implemented.

2. Inoculation methods: The inoculum for each strain was prepared as an aqueous suspension of 2×10^4 urediniospores mL^{-1} according to the methods developed by Ruiz et al. (1989). For inoculations, this spore solution was spray-inoculated onto abaxial and adaxial leaf surfaces of all leaves to the point of runoff for all seedlings in the plot. To assure that the urediniospores used were viable, two *S. jambos* cuttings were utilized as susceptible controls in each block. After inoculation, plants were kept in a mist chamber at $25 \pm 2^\circ\text{C}$ for 24 h in the dark, and subsequently transferred to a growth chamber at $22 \pm 2^\circ\text{C}$ with a 12-hour light cycle (Ruiz et al. 1989).

Rust resistance/susceptibility was evaluated for each inoculated plant at 20 days post-inoculation. The two most infected leaves on each plant were categorized for the amount of disease development they showed according to Jungmans et al. (2003): S0 = immunity or hypersensitive reaction (HR); S1 = punctiform pustules <0.8 mm in diameter; S2 = medium pustules from 0.8 to 1.6 mm in diameter; and S3 = large pustules >1.6 mm in diameter and, in some cases, where pustules developed on the leaf petioles and young branchlets. The term “fleck” was also used to characterize the immune reaction that was occasionally observed. Flecking occurs when localized leaf cells become chlorotic, but a small black spot, which would be indicative of a hypersensitive reaction, does not develop. A seedling was considered resistant if it had been scored S0 or if it exhibited flecking; seedlings scored as S1, S2 or S3 were considered susceptible.



Figure 2. Photo shows all the ohia seedlings in one main plot of one block of the experiment being inoculated with one strain of *Puccinia psidii*.

3. QUANT Image Processing Software (Vale et al. 2003) was used to evaluate the percentage of leaf area covered by rust, based on digitized images of infected leaves (Figure 3).

The split-plot experiment resulted in an unbalanced randomized block design due to some seedling mortality (for a variety of reasons, all seedlings of family 12 inoculated with strain 5 died in Blocks II and III and all seedlings of family 8 which had been inoculated with strain 5 died in Block II). For this reason, the usual ANOVA approach was modified, and

a different mixed general linear model (McCullagh and Nelder 1991) was used instead for the statistical analysis. The combination of the inoculum strain and ohia family (IS-F) was viewed as a categorical fixed effect (with $5 \times 6 - 2 = 28$ levels), with block (B) and the interaction of the block by strain- family combination ($B \times IS-F$) as the random effects. To examine the effects of interest, and compare the strains levels, contrasts were used from the combination IS-F. The maximum likelihood ratio test and the Bonferroni's adjustment for multiple pairwise comparisons (for an experiment-wise $\alpha = 0.05$) were used for testing the contrasts. The SAS v.9.3 MIXED procedure (SAS Institute Inc. 2002-2010, Cary, NC, USA) was used to estimate the means and test the comparisons. Using this approach, statistically valid inferences could be made about each of the following:

A) The mean severity of infection caused by each of the *P. psidii* strains and the standard error; B) The mean severity of infection for each ohia family and the standard error; C) Differences among disease severities caused by *P. psidii* strains; D) Differences in disease severity among ohia families; and E) Differences in disease severity caused by different *P. psidii* strains on different ohia families.

Influence of Severe Infection by P. psidii on Growth and Survival of Ohia Seedlings

To evaluate the impact of severe rust disease on height growth and mortality rates of ohia seedlings, 32 10-month-old seedlings of each of three OP families of ohia (Families 5, 7 and 8) were used. Sixteen of these 32 seedlings for each family were inoculated with the Strain 1 (UVF2) of *P. psidii* in accordance with the same methodology described above. The other 16 seedlings of each family served as control plants, which were not inoculated with a rust pathogen, but were sprayed with 1 mL/L of the rust fungicide trifloxystrobin +tebuconazole (Nativo, Bayer ®). Additional sprays of Nativo were applied to the non-inoculated, control seedlings every 15 days. This experiment was conducted in a completely randomized design in a greenhouse. Lateral branches were pruned during the subsequent growth period. Height growth in millimeters and survival were recorded for all 96 seedlings at 30-day intervals over the next 180 days. At the end of this period, the Area Under the Plant Growth

Curve (AUPGC) was calculated using the method of Shaner and Finney (1977).

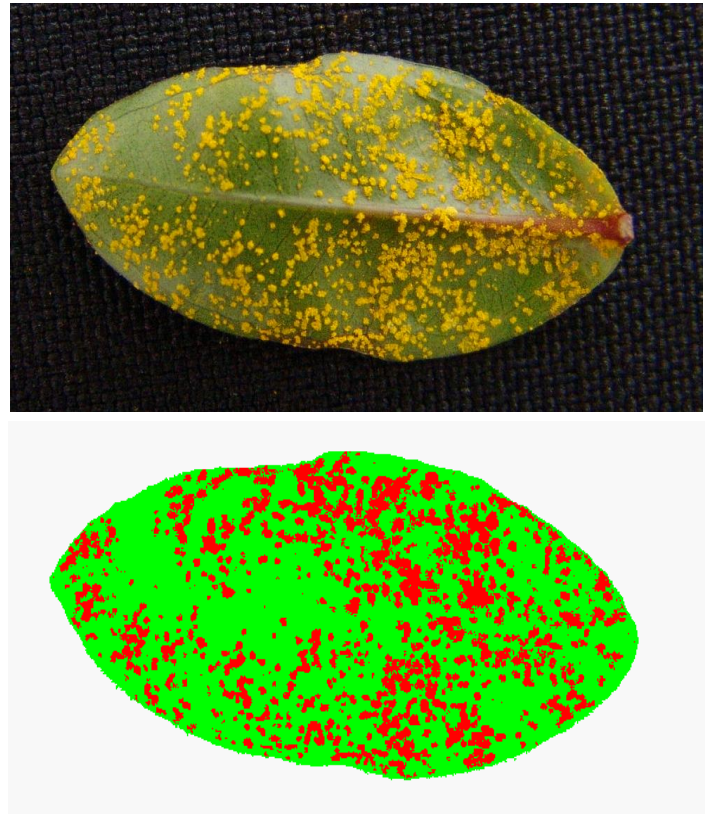


Figure 3. Photo of an ohia leaf infected by *Puccinia psidii* and the image of this same leaf after transformation by the QUANT Image Processing Software (Vale et al. 2003). In this example, 26.65 percent of the area of the leaf was colonized and this was recorded as the disease severity for this leaf.

RESULTS

Influence of Puccinia psidii Strain and Ohia Family on Rust Severity

The results from the inoculation test with the five *P. psidii* strains and six different ohia families illustrate convincingly that three of the strains used in this test, Strain 1 (UVF2 derived from *E. grandis*), Strain 2 (EUBA1 derived from *E. grandis*), and Strain 3 (derived from *M. cauliflora*), are highly virulent on all six of the ohia families used in this test, while Strain 4 (derived from *P. guavaja*) and Strain 5 (derived from *P. araca*) have a significantly lower virulence on these families (Tables 1 and 2). The comparison of calculated P-values with Bonferroni alpha values (Bonferroni's adjusted alpha value determinations ($=0.05/10= 0.005$)) to test the hypotheses of equality for the data (Table 2) show

no statistically significant differences among the infection severities caused by Strain 1 (UVF2), Strain 2 (EUBA1) and Strain 3 (derived from *M. cauliflora*). Furthermore, no significant differences were observed in disease severity caused by *P. psidii* Strain 4 and Strain 5. However, these Bonferroni values also show that highly significant differences ($P=0.0001$) exist between the disease severity caused by the highly aggressive group (Strains 1, 2, and 3) and the weakly pathogenic group (Strains 4 and 5).

No statistically significant differences in disease severity were observed among the six different ohia families for each strain (Table 3). The calculated Bonferroni adjusted alpha value of $0.05/15=0.0033$ had an experimental wise error rate = 0.05. Because none of the comparisons were significantly different at the $\alpha=0.0003$, the hypothesis of equality could not be rejected.

Table 1. The percentage of resistant ohia plants in response to inoculation by five different Brazilian strains of *Puccinia psidii*.

<i>Puccinia psidii</i> strain number	Percentage of resistant Ohia seedlings*
1 (UVF2; derived from <i>E. grandis</i>)	0
2 (EUBA1; derived from <i>E. grandis</i>)	7
3 (derived from <i>M. cauliflora</i>)	0
4 (derived from <i>P. guajava</i>)	61
5 (derived from <i>P. araca</i>)	84

*83 to 86 ohia seedlings were tested for each *P. psidii* strain.

Influence of Severe Infection by P. psidii on Growth and Survival of Ohia Seedlings

Surviving seedlings inoculated with *P. psidii* Strain 1 (UVF2) had, on average, slightly less than one-third the height growth of uninfected seedlings from the same families at 6 months post-inoculation (Table 4 and Figure 4). T-tests showed that this difference was statistically significant at the 0.0001 level of probability for families 5 and 8 and at the 0.005 level of probability for Family 7. Analyses also indicate that the inoculated seedlings had a 27 percent greater chance of mortality

over this same period; compared to non-inoculated, control seedlings.

Table 2. The average disease severities caused by five different *Puccinia psidii* strains on six different ohia (*Metrosideros polymorpha*) families at 20 days post-inoculation.

<i>P. psidii</i> strain	No. of Ohia families	Mean leaf damage %	Std. error of mean leaf damage %
Strain 1 (UVF2)	6	18.1 a ¹	2.0
Strain 2 (EUBA1)	6	14.4 a	1.5
Strain 3	6	15.3 a	1.4
Strain 4	6	4.1 b	1.6
Strain 5	4	3.0 b	1.8

¹Means followed by the same letter are not significantly different from each other.

Table 3. Average disease severity on leaves of six different ohia (*Metrosideros polymorpha*) families in response to different *Puccinia psidii* strains at 20 days post-inoculation.

Family	No. of strains of <i>P. psidii</i>	Mean leaf damage %	Std. error leaf damage %
1	5 (Strains 1,2,3,4,5)	11.5	4.3
5	5 (Strains 1,2,3,4,5)	9.7	3.5
7	5 (Strains 1,2,3,4,5)	10	3.2
8	4 (Strains 1,2,3,4)	10.3	3.4
11	5 (Strains 1,2,3,4,5)	11.9	1.7
12	4 (Strains 1,2,3,4)		4.2

Table 4. Impact of severe disease caused by *Puccinia psidii* (Strain 1; UVF2) on growth (calculated by measuring the Area Under the Plant Growth Curve; AUPGC) and survival of three families of ohia (*Metrosideros polymorpha*) seedlings at 180 days post-inoculation.

Ohia Family	Growth of non-inoculated seedlings		Growth of inoculated seedlings	
	AUPG C	Survival %	AUPG C	Survival %
5	354	69.0	79	44.0
7	762	56.0	245	19.0
8	530	50.0	199	31.0
Averages	549	58.3	174	31.3

DISCUSSION

The salient results show that three *P. psidii* strains, Strain 1 (UVF2 derived from *E. grandis*), Strain 2 (EUBA1 derived from *E. grandis*), and Strain 3 (derived from *M. cauliflora*), used in this test were highly virulent on seedlings representing all six of the ohia (*Metrosideros polymorpha*) families used in this experiment. Furthermore, two *P. psidii* strains (Strain 4 derived from *P. guavaja* and Strain 5 derived from *P. araca*) were much less virulent on these same ohia families.

These results clearly demonstrate that some *P. psidii* strains/genotypes, which are not yet present in Hawai'i, potentially pose a devastating disease threat to native ohia in Hawai'i should these strains be introduced. Thus, a case could be made to implement actions to avoid the introduction of new *P. psidii* strains into Hawai'i. None-the-less, it is a complex regulatory process to establish quarantine regulations in states of the USA for pathogenic species when at least one strain of that pathogenic species has already been found inside that state. Hopefully, the appropriate regulatory authorities in the Hawai'ian Department of Agriculture (HDOA) and Animal and Plant Health Inspection Service (APHIS) will consider the results of this study and others when developing appropriate protection and quarantine regulations to prevent the introduction of new *P. psidii* strains to Hawai'i via incoming myrtaceous plant materials.

Recently, *P. psidii* isolates have been collected on different Myrtaceous hosts from Uruguay, Paraguay, Costa Rica, Puerto Rico, Florida, Mexico, and Australia. Genetic characterization and virulence tests are needed to determine the invasive threats posed by these other sources of this rust pathogen as well.

To date, only one genotype of *P. psidii* has been found in Hawai'i (Zhong et.al. 2008; Kadooka 2010; Graça et al. 2011a). Because of potential invasive threats to Brazil, the Hawai'ian genotype was not included in our virulency tests of Brazilian *P. psidii* strains on ohia. Plans to conduct virulency tests in a controlled biological containment facility that compares Hawai'ian and Brazilian *P. psidii* genotypes are currently under consideration.

Although not statistically significant, data show trends that indicate potential variation in resistance/susceptibility among ohia families inoculated with the same strain of *Puccinia psidii*. Perhaps more natural family-level variation in resistance/susceptibility to different *P. psidii* strains could be detected in an inoculation test using more ohia families that represent more geographically diverse provenances.

Natural variation in resistance/susceptibility to diverse *P. psidii* strains has long-term implications for ohia population dynamics should different *P. psidii* strains become established in Hawai'ian forests; seedlings developing from more resistant parents could be expected to have a better chance of survival.

This natural variation could also be exploited by planting ohia sources that have been selected or deliberately bred for increased resistance to known *P. psidii* strains. The Brazilian programs to develop *Eucalyptus* species, hybrids, and clones that are resistant to *P. psidii* have been especially successful (Alfenas et al. 2009), and could serve as a valuable model in Hawai'i for increasing resistance of ohia and other native species to *P. psidii*. However, because relatively few (ca. 10,000s) ohia seedlings are currently planted annually in Hawai'i, an increase in ohia planting is perhaps needed to justify the costs of an effective breeding program for disease resistance.

The use of leaf photos and QUANT Image Processing Software (Vale et al. 2003) to measure infected-leaf-

area was very effective for obtaining a quantitative measure of infection level. This approach contributed greatly to the provision of precise quantitative data (Tables 2 and 3) that were conducive to robust statistical analyses. However, it should be noted that this measurement could greatly underestimate amount of leaf mesophyll that is infected by *P. psidii*. Anatomical examinations show that *P. psidii* mycelia can occupy a leaf area in the mesophyll that is two to three times larger than the area occupied by the uredia on the leaf surface (Janice Uchida pers. com.*). In some of the split plots, as much as 33 percent of the leaf surface of ohia plants was occupied by uredia (Table 2); this would translate to somewhere between 66 percent and 99 percent of the mesophyll of these leaves having been infected by *P. psidii* mycelia.



Figure 4. The growth of *Metrosideros polymorpha* seedlings was greatly affected by a severe infection of *Puccinia psidii*. The seedling on the right was heavily inoculated with *P. psidii* urediniospores under conditions ideal for infection, and the seedling on the left was not inoculated. Both seedlings were then maintained in suitable greenhouse under conditions ideal for their growth for 180 days.

In the growth and mortality impact study, height growth of inoculated seedlings was only 31 percent of the

height growth of non-inoculated seedlings of the same family. Thus, *P. psidii* infection can have a tremendously deleterious impact on growth of ohia seedlings. Furthermore, *P. psidii* infection hugely reduced survival of ohia seedlings, with a 27 percent greater level of mortality compared to non-inoculated seedlings of the same family (Table 5). In other controlled experiments, many rust pathogens have demonstrated significant negative growth impacts on diverse plant hosts (Dianese et al. 1978; Geils and Jacobi 1993; Mwando et al. 2012; and Wennstrom 1999) but the growth impacts of *P. psidii* shown in this experiment are generally greater than those demonstrated in most of these other experiments.

The high impact of *P. psidii* on growth and survival in this experiment is perhaps related to the virulence of the *P. psidii* strain used (Strain 1; UFV2), but may also be attributed to the very conducive inoculation conditions and high inoculum densities used in this experiment. The inoculation methods used in this experiment are the same ones Brazil uses to screen *Eucalyptus* clones for resistance to *P. psidii*. Because of the huge economic considerations associated with *Eucalyptus* plantations in Brazil, extremely rigorous screening programs are in place in this country to assure that deployed *Eucalyptus* clones are extremely resistant to *P. psidii*.

CONCLUSIONS

These studies conclusively demonstrate the existence of *P. psidii* strains, not yet in Hawai'i, that can be highly aggressive on ohia. However because the conditions for these tests (high inoculum levels, high air moisture content and ideal temperature) were so conducive for infection, it is not possible to predict the epidemiological behavior that these same strains might show in the natural ohia forests of Hawai'i nor the exact ecological ramifications that might result should they be introduced into this state. Nevertheless, it seems especially prudent to avoid this threat by any practical means.

Despite the fact that one strain of *Puccinia psidii* already exists in Hawai'i, our results indicate that other strains of this fungus continue to pose a potential threat to the ohia forests of Hawai'i should they become introduced. This information lends support to improving state and federal restrictions to reduce the risk of future

incursions of additional strains of *Puccinia psidii*. The recognition of the variability in virulence of different strains of *Puccinia psidii* should be useful towards articulating effective biosecurity policies.

These studies show that three out of five *Puccinia psidii* strains from Brazil are highly pathogenic on ohia (*Metrosideros polymorpha*) seedlings under experimental conditions. Although this experiment was conducted explicitly to explore the risks that other strains of *Puccinia psidii* might pose to the ohia of Hawai'i, the myrtaceae family contains over 4000 species and, indeed, is one of the largest tree families in the world. As such, the results from this study can serve as a baseline for studies on Myrtaceous species from other parts of the world that are facing similar invasive threats from *P. psidii*.

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CONTRIBUTED PAPERS





CLIMATE CHANGE DRIVEN MORTALITY OF DOUGLAS FIR NEAR ITS MOST SOUTHERLY AND LOWER ELEVATION RANGE LIMITS

Martin MacKenzie¹

ABSTRACT

A USFS Forester on the Groveland District of the Stanislaus National Forest in California reported what appeared to be a high level of Douglas fir mortality in 2008. In 2009 twenty, one-tenth acre permanent sample plots were established in this area to document more accurately what was happening to Douglas fir at this site. This site is at an elevation of 4,000 ft above sea level, which is low for Douglas fir at this latitude. Also present on this site were ponderosa pine, and incense cedar and a few sugar pine. Only one to two trees per acre had died in each of the previous 15 to 20 years. However, Douglas fir accounts for 97 percent of the dead stems and 99 percent of the dead basal area even though it only comprises 40 percent of the live basal area of these stands. Symptoms of black stain root disease were very rarely observed in the apparently declining Douglas fir, but the California flat-headed borer was omnipresent in the dead trees. Observations of higher elevation Douglas fir stands on the adjacent Ranger Districts revealed no Douglas fir decline. Beneficiaries of this decline (in the short run) are the ponderosa pine and the occasional sugar pines, with the shade tolerant incense cedar likely to be the eventual victor. This appears to be a disease driven species conversion and is best viewed as a local species decline. Invoking Manion's "Decline Spiral" the situation is best explained as a Climate Change Scenario, in which; Climate Change is the **Predisposing factor** that "*cocks the gun*", a persistent drought is the **Inciting factor** that "*pulls the trigger*" and Black Stain Root Disease along with the California Flat headed borer are the **Contributing factors** ("*the bullets*") that kill the Douglas fir.

INTRODUCTION

Often, the areas of mortality that get entered into pest conditions reports are those that are plainly visible from the roadside. Were it not for systematic aerial detection flights by trained observers pest detection reports might mainly reflect the serendipitous drive-by detections of windshield observers. Furthermore, the areas of damage that get most attention are the massive ones arising from wildfires or bark beetle outbreaks. Frequently when the mortality amounts to no more than one to two trees per acre it gets attributed to being a "healthy amount" of nothing more than "background mortality".

When the forester Jim Serra (Groveland Ranger District, Stanislaus National Forest) was marking roadside hazard trees, in preparation for a thinning operation, he concluded that there were an "unhealthy" number of dead Douglas fir trees in the stand he was marking. A review of previous aerial detection flight records and a special flight to photograph the stand in

question (Figure 1) revealed that there appeared to be no more annual mortality than what could be considered "background".



Figure 1. The image that led to the tree health survey. An aerial image captured on the 2008 FHP aerial flight over the Stanislaus National Forest. Notice that there are no large standing dead trees along the tree edge of the road. The GPS'ed flight path of the spotting aircraft gave the general location of this image and Ranger District staff found 3 points of confirmation. Once the image had been tentatively located the crowns of, and shadows cast by individual trees could be located on the ground.

In: Browning, J. Comp. Proceedings of the 60th Annual Western International Forest Disease Work Conference; 2012 October 8-12; Tahoe City, CA.¹USDA, Forest Health, Sonora, CA.

OBJECTIVE

The present study was initiated to explain the dichotomy between, the level of tree mortality in a stand that a ground based forester considered having an “unhealthy level” of Douglas fir mortality and mortality level that aerial detection would suggest is no more than “normal background mortality”. For the purposes of this discussion “normal background mortality” is taken to equal the level of annual mortality needed in a normally stocked stand as the residual trees grow from one diameter class into the next class.

METHODS

During the summer of 2009, ten, one tenth acre circular plots were distributed over the area covered in Figure 1, and another ten plots were evenly distributed over an adjacent part of the project area that had forest road 1S68 running through it. Within each plot all stems greater than 5 in. were tallied. It quickly became apparent that the California Department of Transportation (Caltrans) has been in a habit of felling all dead trees that were once located within striking distance of Highway 120 (the access road from Groveland to Yosemite National Park). This observation necessitated dividing trees into three categories, standing living, standing dead, and felled dead. Breast Height Diameter (BDH) was recorded for all stems and when observed, the symptoms of characteristic of Black Stain Root Disease (BSRD) were recorded along with all signs and symptoms of insect and other fungal attacks. Each plot had its center marked with a redwood stake and its GPS coordinates were recorded. Each tree within the plots was tagged with an aluminum tree tag (affixed with an aluminum nail). As it is intended that these plots will be reassured periodically, maps were drawn up to aid in plot relocation and all data has been archived in FHP files.

Previously, retired FHP pathologist John Pronos had remarked that the stands of the Middle Fork Project contained the most southerly known location of Black Stain Root Disease in the native range of Douglas fir. BSRD is caused by the fungus *Leptographium wagneri* var *pseudotsugae* (Jacobs and Wingfield

2001). Initial investigations, in the marked (for thinning) stands along Forest Road 1S68, confirmed the likelihood that the situation was BSRD mortality of Douglas fir. As the fungus was induced to fruit on chips taken from symptomatic roadside Douglas fir saplings a major effort was made to find symptomatic plot trees. Although, service area files and local knowledge have never recorded the Douglas fir beetle being present in the Groveland RD an attempt was made to trap this insect. As initial examinations revealed a flat headed borer in dying and recently dead fir trees, insects were reared from bark slabs of dead, non-plot, trees marked for thinning.

RESULTS

The results from all 20 of the plots evaluated in this study are summarized in Table 1. The mortality distribution is given in Figure 2. Of the 29 dead trees encountered in the plots only one was not a Douglas fir; a 9.8 in. *Pinus ponderosa*. Douglas fir, which accounted for 40 percent of the living basal area, also accounted for 97 percent of the dead stems and 99 percent of the dead basal area. For every 2 square foot of living Douglas fir basal area there was 1 square foot of dead basal area. The average diameter of the dead Douglas firs was almost 2 in greater than the average diameter of the living trees, 16.7 vs. 18.6 in.

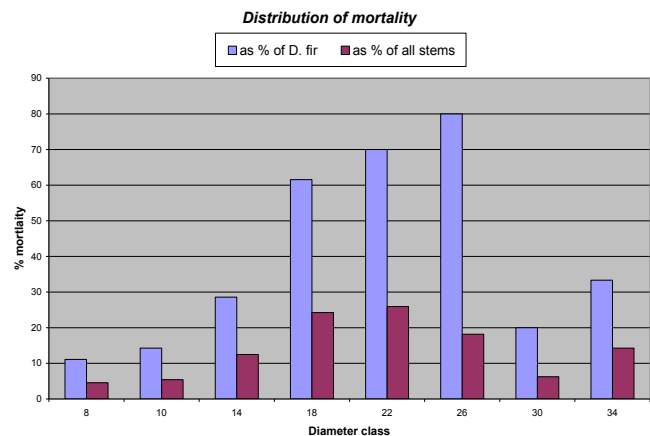


Figure 2. The percentage mortality of either all stems in a diameter class or of only the Douglas fir diameter classes. (Note the same dead trees are used to generate both graphs; however, all but one of the dead trees was a Douglas fir).

Table 1. Survey data summary, with basal areas in sq ft/ acre. Total trees on plots is presented to avoid having to present data as partial trees per acre.

	Basal Area		Trees on Plots		Spa*	
	Living	Dead	Living	Dead	Living	Dead
Douglas fir	62.09	30.19	67	28	33.5	14.0
Ponderosa pine	30.47	0.26	17	1	8.5	0.5
Incense cedar	34.48		43		21.5	
Black oak	16.86		15		7.5	
Sugar pine	9.98		14		7.0	
Interior live oak	0.13		1		0.5	
Class total	154.01	30.45	157	29	78.5	14.5
Grand total	184.46		186		93	

Trapping in 2009 and 2010 failed to detect either the Douglas fir beetle (*Dendroctonus pseudotsugae*) or the fir flat headed borer (*Phaenops drummondi*). However, after the on-site chipping of thinning residues, the Douglas fir bark beetle was trapped (Spring of 2012). The insect most frequently reared from the bark slabs, identified by CDFA's C. Bellamy, was the Californian flat headed borer (*Phaenops californica*).

DISCUSSION

In a retrospective article presented at an American Phytopathological Society sponsored symposium Professor Paul Manion introduced, to the wider group of forest pathologist, his contention that “forests need a healthy amount of disease” (Manion 2003). In light of the normal volume tables proposed for fully stocked stands Manion’s “healthy amount of death” equates to the number of stems that are lost when the stand grows from one age class into the next ageclass. Returning to Figure 1 the question to be answered is; “Do those arrows on that image represent a healthy, normal, or background level of mortality?” From his airborne perch the author estimated the level of mortality at 1 - 2 stems per acre per year. Of the 29 dead trees in the 2 acres of plots only 4 still had needles attached. If some of those trees had been dead for over a year this would equate to a mortality level of 1 - 2 stems per acre per year. If 1-2 stems are dying per year it would take 10-15 years to accumulate a number of dead trees equal to those tallied in this survey. As mentioned earlier, the periodic reassessment of these plots will provide data to confirm or refute this speculation.

At first assessment a mortality rate of 1 - 2 trees, per acre, per year might be an acceptable (healthy) level of

background mortality. However, almost all of the mortality is focused on one species. At this location Douglas fir is in decline and the beneficiaries of this decline (in the short run) are the ponderosa and sugar pines, with the shade tolerant incense cedar likely to be the eventual victor. Incense cedar already has a commanding lead over the pines in number of stems and a slight lead over ponderosa pine in basal area. Death of the Douglas fir amounts to a biological thinning of the ponderosa and sugar pines. This is a thinning in which no pines have to die in order to provide the growing space necessary for the remaining pines, for all the “sacrifice” (mortality) is being made by the Douglas fir.

Figure 2 was constructed to describe where the Douglas fir mortality was occurring. For each diameter class in which there were more than 4 total trees (of all species) the number of dead Douglas firs was expressed as a percentage of either the total diameter class and of just the Douglas fir cohort. In this survey the largest tree was a ponderosa pine at 44.9 in. The second and third largest trees were both Douglas firs, one living (43.6 in) and one dead (41.8 in)! Professor Manion (2003) suggested that there should be “a healthy amount of disease in a healthy forest.” This amount of death is what can be called the background mortality that occurs when a stand develops and its trees grow from one diameter class into the next diameter class. Unfortunately, the science of forest pathology has not progressed to the point where we can quantify what is a; “healthy amount of disease or death”.

Searching for a quantification of a healthy amount of death in a healthy forest Manion worked with sugar maple deaths in the Adirondack forests of NY State. In

their earlier paper Manion and Griffin (1998) found that the mortality needed for any diameter class to grow by 1 in. ranged from 19 to 26 percent depending upon forest type. In a subsequent paper Manion (2003) states that; ...“baseline mortality in forests and for the major tree species is generally around 20 percent per 1 in.-diameter class.” Although the available studies are limited it would appear that baseline mortality (the healthy amount of death in a stand) may well be around 22 percent per decade. Returning to the stands on the Stanislaus NF, it can be seen (Figure 2) that if the mortality is expressed as a percentage of the total stems the stand mortality is close to “baseline”. It is only when the mortality of the Douglas fir is related to the total number of Douglas fir stems that the stand falls into the “unhealthy category”, and even then it is only one species that is in an unhealthy state. The stand is being biologically thinned and the pines are doing “well” at the expense of the Douglas fir.

The identification (by Dr Bellamy) that the buprestid reared from the recently dead trees was Californian flat headed borer (*Phaenops californica*) was unexpected, for this insect is best known for attacking pines. It was expected that the reared buprestid would have been fir flatheaded borer (*Phaenops drummondi*). Besides stating that in the insect usually attacks pines Lyon (1970) states “Most often the insects infests pines growing on shallow or rocky soils in stands at the fringe of forested areas where rainfall is light;” Much of the area of the Middle Fork Project area could easily be described as having a shallow rocky soil. Although the Middle fork area is not on the fringe of the forest it is on the fringe of where Douglas fir grows (at this elevation). Egan (2009) compared the deviation from the 30 year average for the NRCS SNOTEL station at Sonora Pass he found that 8 of the 10 years prior to 2008 had below average precipitation and when he compared the trend line for the three Stanislaus Ranger District stations it was possible to conclude that the trees have been under a water stress for much of the past decade.

Using the Western Regional Climate center’s data base (<http://www.wrcc.dri.edu/>) Meyer and Safford (2012) produced an internal FS white paper entitled “A summary of current trends and probable future trends in climate and climate-driven processes in the Stanislaus National Forest and the neighboring Sierra Nevada.” For this synthesis they used the data from the Cherry and Eleanor lake meteorological stations. The two

stations are less than two miles apart and although about 800 ft higher than the study site they are within the Groveland Ranger District. The graphs of Meyer and Safford (2012) depicting the mean maximum, mean, and mean minimum temperature’s for these two stations, while fluctuating from year to year, show a steady positive trend from 1912 through 2009. These trend lines, simple linear regressions with no transformations, provide the best and most conclusive evidence, currently available, of a warming climate on the Groveland Ranger District of the Stanislaus National Forest.

Ever since Robert Hartig adapted the germ theory to forest pathology (Merrill et al. 1975) forest pathologists have used the disease triangle (or adaptations of it) to explain mortality events involving one host species and one fungal pathogen. By the mid 60s pathologist were not able to fully explain tree decline by using the disease triangle alone. Pathologists frequently found that tree deaths had many contributing factors. This led Sinclair (1965) to describe categories of factors to explain these multi-caused declines. A “Forest Decline Session” held at the 1988 American Phytopathological Society annual meeting was followed by a 1990 joint symposium between the American and Canadian Phytopathological Societies. Out of this joint “Forest Decline Symposium” came the APS publication “Forest Decline Concepts” ed. by Manion and Lachance (1992).

In the first chapter of Manion and Lachance (1992) Houston proposed “A Host-Stress-Saprogen model for Forest Dieback-decline Diseases”. This model was essentially the disease triangle in three dimensions and became the disease tetrahedron. Houston had added time as the fourth dimension to the triangle. Of all chapters in this book it was the last, “Forest Decline Concepts; an overview” by Manion and Lachance that has received the most attention. This chapter included the “Decline Disease Spiral” that had appeared earlier in the textbook “Tree Disease Concepts” (Manion 1991). The decline disease spiral has become known as the Manion Spiral. It was Sinclair (1965) who first divided stressors into three categories; **predisposing factors** (i), **inciting factors** (ii) and, **contributing factors** (iii). The decline spiral predicts that a species can go into decline if one factor from each of the three groups is acting on a species at the same time.

For the purposes of discussion the relevant stressors thought to be active on the on the Douglas fir in this corner of Stanislaus National Forest have been tabulated in Table 2. Douglas fir growing above 5,000 ft, along the Clark Fork of the Stanislaus River appears to be as healthy as those growing at a similar elevation on the Sierra National Forest. The most southerly natural limit of Douglas fir is not on the Stanislaus National forest but on the Sierra National forest, where it grows at nearer to 5,000 ft and where BSRD has never been reported. The observed Douglas fir decline appears to be restricted to lower elevations of the Stanislaus National Forest. Following the Manion model, the Climate Change is viewed as being the predisposing factor, the drought is the inciting factor that triggered the mortality and the contributing factors that lead to tree death are viewed as being the insects and BSRD.

Table 2. The stressors acting upon Douglas fir on the Groveland Ranger Districts of the Stanislaus National Forest tabulated in into the Sinclair Classes as used in the Manion Decline Spiral model, with the major stressors highlight in yellow.

(I) Predisposing factors	(II) Inciting factors	(III) Contributing factors
Climate change	Drought	CA flatheaded borer BSRD
Soil compaction	Cankers	D. fir engraver
Outside climatic range	Defoliating insects	Other bark beetles etc.
Dwarf mistletoe		
Stand structure		

SYNTHESIS

The best model to explain the Douglas fir decline observed here is the Manion Spiral. Using the Manion spiral we can suggest that under the current conditions at the lower elevation and altitudinal limits of Douglas fir on the Stanislaus National Forest the species is declining. Invoking Manion’s “Decline Spiral” the situation is best explained as a Climate Change Scenario, in which; Climate Change is the **Predisposing factor** that “cocks the gun”, a persistent drought is the **Inciting factor** the “pulls the trigger” and Black Stain Root Disease along with the California Flat headed borer are the **Contributing factors** (“the bullets”) that kill the Douglas fir. If this scenario is accepted we now have another predisposing factor, for now Douglas fir at 4,000 ft, in the Southern Sierras is growing outside its climatic range. The long-term

implication of these observations is that under a scenario of climate change Douglas fir is dying out at its lower elevation limit and this scenario amounts to a pest driven change in species composition that allows the stands to remain fully stocked throughout. This example provides support for the rationale of Monitoring at the Margin provided the margins are at the lower elevation and latitude limits of the tree species.

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SIMULTANEOUS CLIMATE ENVELOPE MODELING FOR WESTERN HEMLOCK AND HEMLOCK DWARF MISTLETOE

Tara Barrett¹, Paul Hennon², Greg Latta³, Bianca Eskelson³, and Hailemariam Temesge³

Analysis of weather station data for the temperate rainforest region in Alaska shows mean annual temperature has increased about 1.5 C over 50 years, and general circulation models (GCMs) predict further warming over the next century. We analyzed inventory data and developed climate envelope models to (1) better understand the relationships between western hemlock, hemlock dwarf mistletoe, and climate in the Pacific Northwest and Alaska and (2) predict how potential habitat for hemlock dwarf mistletoe and western hemlock might change over the next century.

Data used for current relationships came from the Forest Inventory and Analysis program (FIA), with 1503 forested plots measured between 2001 and 2007 in California, Oregon, and Washington, and 1549 forested plots measured between 1995 and 2003 in the temperate rainforest region of Alaska. The combined data sets showed an infection rate that peaked at progressively lower elevations as one moves north within the western hemlock range (Figure 1).

Within southeast Alaska, there were also strong indications that climate currently limits hemlock dwarf mistletoe to a subset of the range of western hemlock; dwarf mistletoe is limited to southeast Alaska, while western hemlock's range extends more than 600 km further west to Prince William Sound.

Within southeast Alaska, 20 percent infection was found at sea level, but dwarf mistletoe infection dropped off rapidly to less than five percent infection by 200 m elevation, and was not found in trees above 400 m elevation although western hemlock was found in elevations up to 800 m. When overlaid against the PRISM (PCG 2002) spatial model of climate (1961-1990) in southeast Alaska, western hemlock trees with dwarf mistletoe had an average of 22 additional days above freezing compared to uninfected hemlock trees.

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Table 1. Results for using three different types of modeling approaches to predict hemlock dwarf mistletoe for an independent validation data set. Underlined values indicate best performing model for the accuracy metric.

Accuracy metrics	MSN	Random forests	Logistic
Sensitivity (correct prediction of presence)	24	20	<u>37</u>
Specificity (correct prediction of absence)	90	<u>96</u>	93
Bias (under or over prediction)	<u>2.1</u>	-3.4	n.a.
Range (similarity to map of presence)	Good	<u>Best</u>	Better

Using the presence/absence data with spatial climate information representing growing degree days, solar radians, rainfall, snowfall, evapotranspiration, and minimum average annual, spring, and fall temperatures, we used three types of modeling approaches to forecast how habitat for hemlock dwarf mistletoe and western hemlock might change over the next century. Testing the three modeling approaches against current presence/absence data showed that no single approach did best by all measures of accuracy (Table 1). Using a composite GCM and three different climate scenarios (A1B, A2, and B1), the models projected modest increases in habitat for western hemlock, which reaches the northern part of its range in Alaska. In contrast, the models projected very large, but highly varied, increases in dwarf mistletoe habitat in Alaska over the next century (Figure 2). While dispersal limitations may prevent dwarf mistletoe from occupying projected new habitat in areas such as Valdez or Prince William Sound (Figure 3), there does seem to be a large potential for increase in southeast Alaska, where hemlock dwarf mistletoe is already present. More detail on methods and results can be found in Barrett et al. (2012). More detail on methods and results can be found in Barrett et al. (2012).

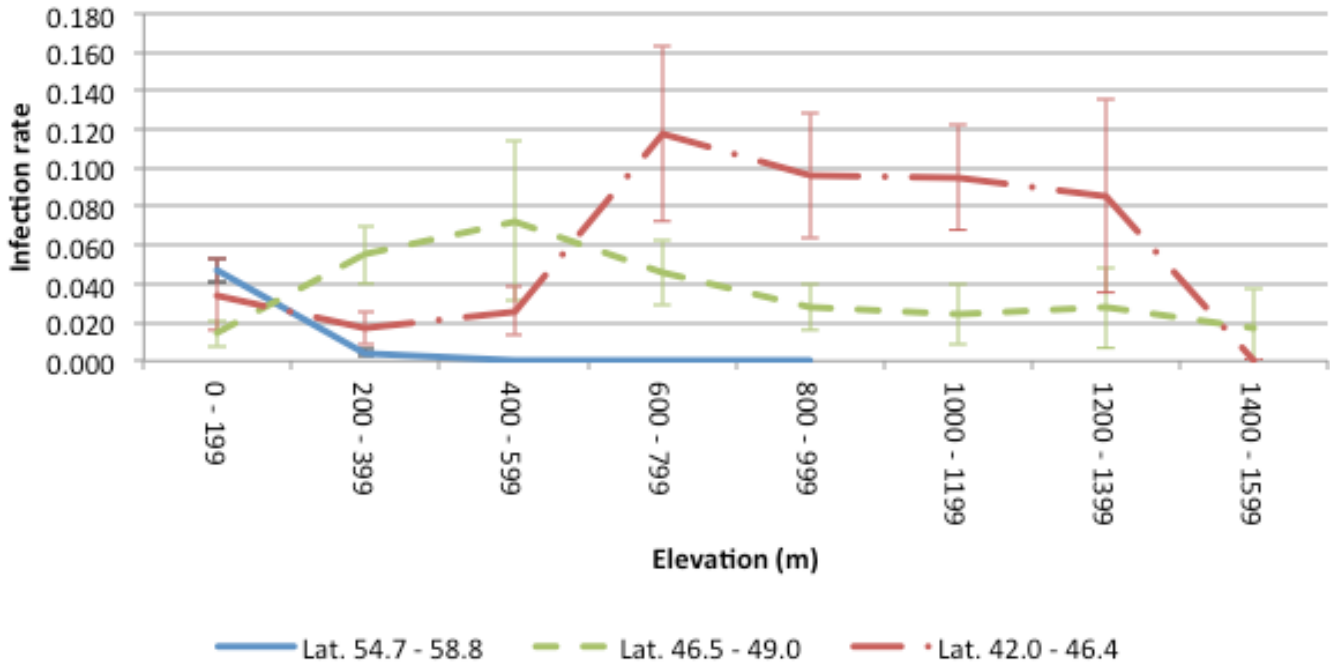


Figure 1. The rate of infection of western hemlock trees by hemlock dwarf mistletoe peaks at progressively lower elevations as one moves from northern California to southeast Alaska.

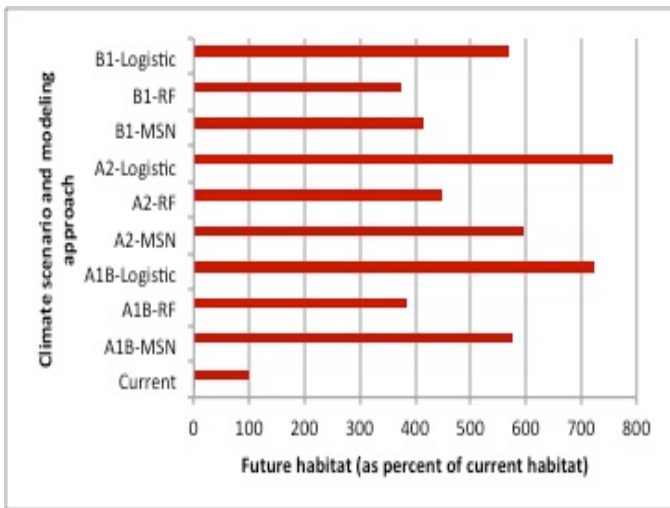


Figure 2. 100-year projections of future dwarf mistletoe habitat in coastal Alaska, by climate scenario and modeling approach.

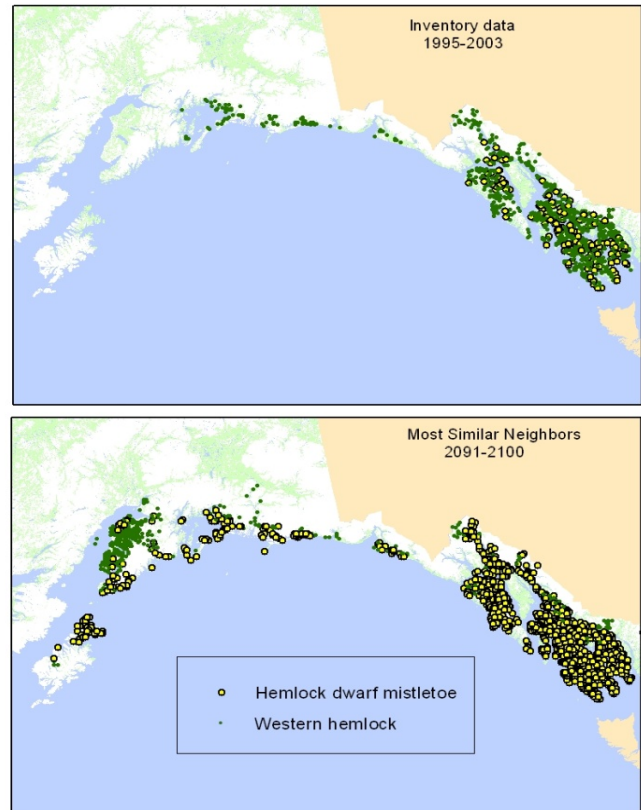


Figure 3. Climate envelope modeling shows substantial increases in potential habitat for dwarf mistletoe (bottom), both in southeast Alaska, where it is currently found (top) and westward along the Gulf of Alaska coast.

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BLISTER RUST UPDATES ON RESISTANCE AND FIELD TRIALS: ON THE ROAD TO DURABLE RESISTANCE? A TREE BREEDER'S PERSPECTIVE

Richard A. Sniezko¹

INTRODUCTION

Genetic variation represents the key building block of life and evolution for all species, including forest trees. The genetic variation that exists within a forest tree species is the underlying basis for tree improvement programs for increasing growth in managed forests. Within-species genetic variation is also the source of genetic resistance to insects and pathogens in forest trees and for buffering a species against a changing climate and the associated abiotic stresses. A number of programs exist that seek to raise the frequency and level of genetic resistance in our native tree species to pathogens or insects (King and others 2010; Sniezko 2006; Sniezko and others 2012a).

White pine blister rust, caused by the non-native, invasive pathogen *Cronartium ribicola*, is wide-spread in western North America and has been found on seven of the eight species of white pines that are present in the western United States (<http://www.fs.fed.us/rm/higherelevationwhitepines/Threats/blister-rust-threat.htm>; Schwandt and others 2010). In the Pacific Northwest Region (USFS Region 6; Oregon and Washington) white pine blister rust has caused extensive mortality in the three white pine species that it impacts: sugar pine (*Pinus lambertiana*), western white pine (*P. monticola*) and whitebark pine (*P. albicaulis*). These three species are extremely susceptible to the rust. In many areas of OR & WA (the focus of this presentation and paper), even some sites rated as low rust hazard can show high levels of rust infection on trees within 20 years of planting (Sniezko and others 2012d).

Genetic resistance to the rust, coupled with silvicultural tools such as pruning or appropriate site selection are needed to ensure the white pine species will meet management objectives for reforestation and restoration and be maintained as components of our forest ecosystems. Operational programs are underway in the

Pacific Northwest to develop populations of western white pine, sugar pine and whitebark pine genetically resistant to white pine blister rust (Kegley and Sniezko 2004; King and Hunt 2004; McDonald and others 2004; Sniezko and others 2011, 2012b). The Region 6 programs are based at Dorena Genetic Resource Center (Figure 1), a regional facility that is part of the USDA Forest Service's Genetic Resource and Forest Health Protection programs.



Figure 1. Blister rust resistance testing of various white pine species at Dorena GRC (Photo: R.Sniezko).

RUST RESISTANCE IN ARTIFICIAL INOCULATION TRIALS

The program to develop genetic resistance to blister rust in western white pine in Region 6 has been ongoing for more than 50 years. Progenies of over 4500 western white pine parent trees selected in forest lands of a wide array of landowners have been evaluated for resistance through artificial inoculation of seedlings (Figure 2) (Kegley and Sniezko 2004; McDonald and others 2004). The focus in this paper is on western white pine, but progenies of thousands of parents of sugar pine and hundreds of parental selections of whitebark pine as well as numerous parents of *P. strobiformis*, *P. flexilis*, *P. aristata*, and *P. longaeva* (the latter three in conjunction with Dr. Anna Schoettle, RMRS) have also been tested for rust resistance at Dorena GRC (see

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publications at www.fs.usda.gov/goto/r6/dorena, Sniezko and others 2011, 2012b, or contact Dr. Schoettle or the author for details). The progenies of most of the forest selections show extremely high infection and mortality in seedling testing. However, a number of types of resistance have been found, including types of complete resistance (Kinloch and others 1999, 2003) and partial resistance (Kegley and

Sniezko 2004; King and others 2010; Sniezko 2006; Sniezko and Kegley 2003a, 2003b). The operational programs aim to select trees for testing, identify the various types of resistances in seedling progeny in artificial inoculation trials, establish field trials to validate the resistance, and increase the level and frequency of resistance available for reforestation and restoration.

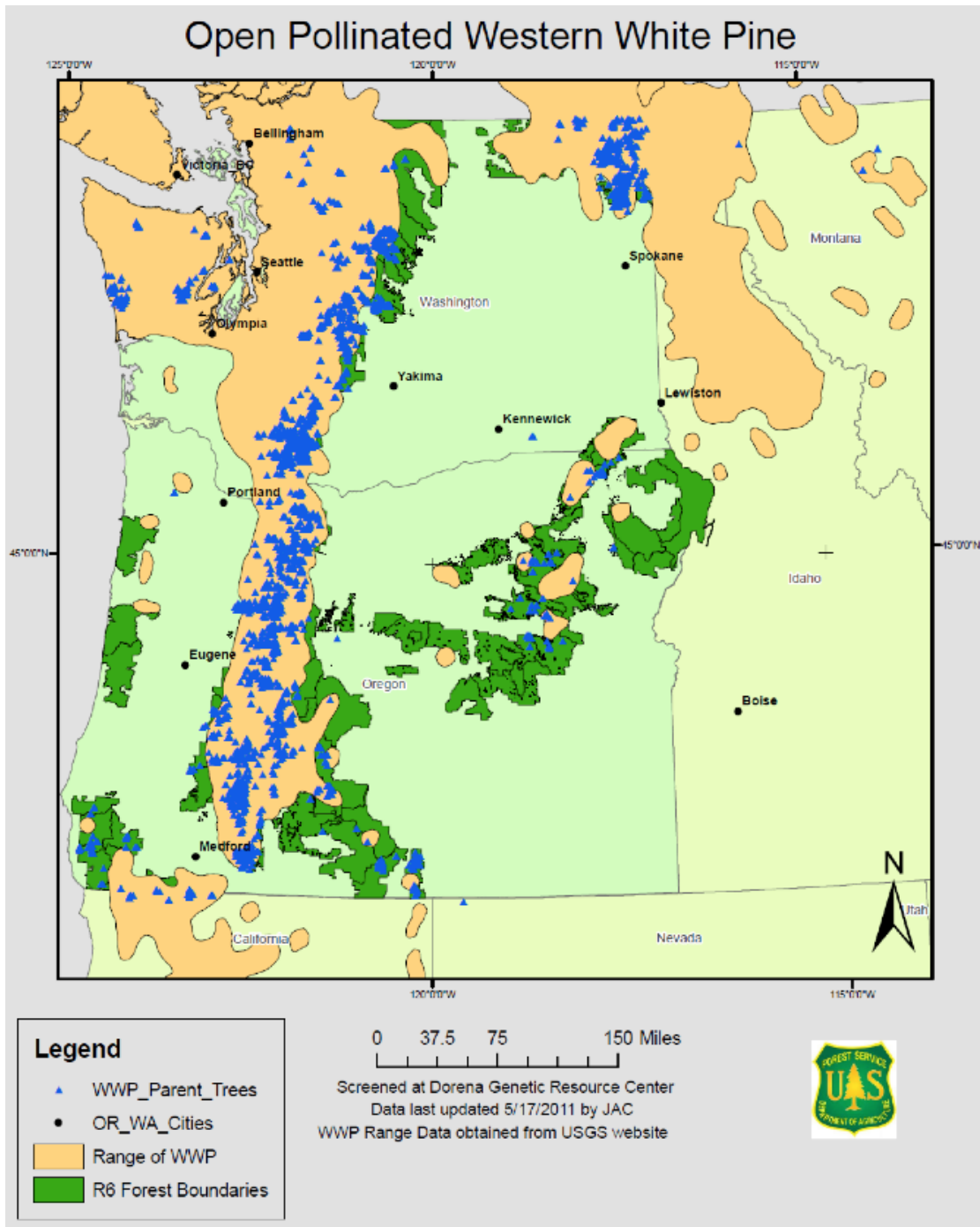


Figure 2. Geographic sources of western white pine parent trees whose seedling progenies have been tested for white pine blister rust resistance in artificial inoculation trials.

The complete resistance in western white pine that is most well documented is controlled by a single, dominant (major) gene (Cr2). It occurs at very low frequency in natural populations and has been found in some forest ecosystems only in Oregon, California and southern Washington (Kinloch and others 1999, 2003; Sniezko, unpublished data). This resistance generally restricts the fungus to the needle, preventing stem infection, and is recognized by a brown band of necrotic tissue encircling the yellow needle lesion (Danchok and others 2012). Although this resistance has been characterized as a hypersensitive-like response (HR) in the needles, further investigation is underway to clarify the underlying nature of this resistance (Sweeney and others 2012). Parents with the Cr2 gene for resistance produce progeny that can show very high levels of resistance, 50 to 100 percent canker-free trees, in inoculation tests or field trials. Despite the dramatic resistance conveyed by HR, this resistance can be overcome by virulent genotypes of the rust, and for western white pine virulent vcr2 rust genotypes have been found in high frequency in several areas in eastern Oregon (for example Figure 3)(Kinloch and others 2004; Kolpak and others 2008; Sniezko and others 2012b).



Figure 3. Western white pine trial (at BLM's Tyrrell seed orchard complex) in fall 2012. The row plot with extensive mortality is a family with 'major gene resistance' from a hypersensitive-like response (HR) infected and killed by virulent vcr2 genotypes of the rust, contrasted with the lower infection levels in the adjacent families with partial resistance (Photo: R.Sniezko).

Although more difficult to evaluate, the main emphasis in the Pacific Northwest Region is developing populations of western white pine with high levels and frequency of partial resistance (Figure 4) (Kegley and Sniezko 2004; Sniezko and Kegley 2003a, 2003b, 2006). Partial resistance may restrict the fungus from entering the stem, reduce the number of needle and/or stem infections, lengthen the time period from needle infection to stem infection, or wall off or slow the growth of the rust fungus in the stem (Figure 5).



Figure 4. Western white pine (WWP) select tree (21105-052) from the Colville National Forest. This is one of the most tested WWP parent tree to date and its wind-pollinated progeny shows a good level of partial resistance (Photo: Brock Mayo).

These resistances have principally been documented in artificial inoculation trials of young seedlings, often at low levels in a family and low frequency in natural populations. A small number of seedlots from the Forest Service Region 1 and British Columbia rust resistance programs have also been examined in trials at Dorena GRC and show resistance phenotypes similar to those found within the range of the selections from OR & WA (Kegley and Sniezko 2004; Sniezko, unpublished), with the exception that no HR type resistance has been noted from those areas. Seedling families from a subset of the Region 6 western white pine parent tree field selections have been evaluated numerous times to verify

the resistances and examine their efficacy with differing geographic sources of rust, inoculum densities or environmental conditions (Figure 4). Field validation of these resistances is well underway. Screening of some of the first control crosses of advance-generation selections is also underway and some of these crosses show high levels of resistance (Figure 6).



Figure 5. Western white pine tree with 302 bark reactions at the Optical field trial on Cottage Grove Ranger District, Umpqua National Forest (Photo: R. Sniezko).

RUST RESISTANCE IN FIELD TRIALS

Short-term testing using artificial inoculation of seedlings can be an invaluable tool, but only if the results relate well to field resistance. Field trials are essential to validate the findings of seedling screening trials as well as to monitor durability of resistance under sites of various rust hazards and changing climate. The Pacific Northwest Region has used individual families (half-sib, full-sib, or selfed progeny of known parents) as well as bulked orchard seedlots to establish a large number of field trials since 1996 (Figure 7)(also for some results see Kolpak and others 2008; Sniezko and others 2004, 2012b). Field trials that follow resistance of individual families allow us to better discern which specific resistances are most effective in the field and portend best for durability of resistance whether used in managed forests or restoration efforts (Figure 3). Most of the early field trials used some of the best wind-pollinated seedlots from field selections for testing, but

the most recent trials have incorporated some of the early advanced-generation crosses as well. Several of the most recent field trials established (and others planned for 2013 and 2014) in Washington and British Columbia have incorporated resistant families and orchard seedlots from operational resistance programs in Forest Service Regions 1 and 6 as well as British Columbia. These trials contain the most diverse set of resistance mechanisms known to date. A subset of these field trials will need to be followed long-term to evaluate the durability of resistance.

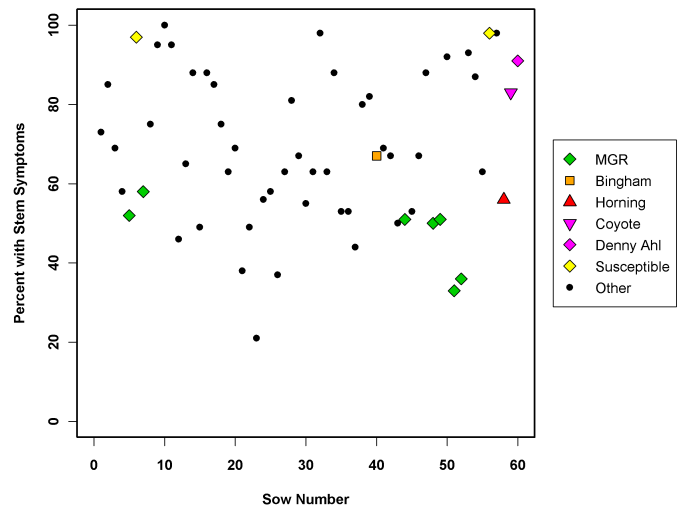


Figure 6. Percent of western white pine seedlings for 56 family and four orchard seedlots (coded here as Sow Numbers) with stem infections ~15 months after artificial inoculation with *Cronartium ribicola* at Dorena GRC. The trial is still ongoing, but shows the very high early infection level of the two susceptible control families as compared to the other families and orchard bulk lots in the trial that have differing levels and types of resistance (major gene resistance or partial resistance).

The early results from the field trials are encouraging. Several of the trials are on sites showing high levels of rust (where >90 percent of the susceptible controls are infected, and those are the ones discussed here). As expected, based on the results of seedling testing using artificial inoculation, there is a wide range in the percentage of trees in a family that become infected, but on sites of higher rust hazard all families show a moderate to high level of infection (for example, see Figures 8 & 9; also Kinloch and others 2008; Kolpak and others 2008; Sniezko and others 2004; 2012b). The susceptible control generally shows the highest or among the highest infection percentage at all sites.

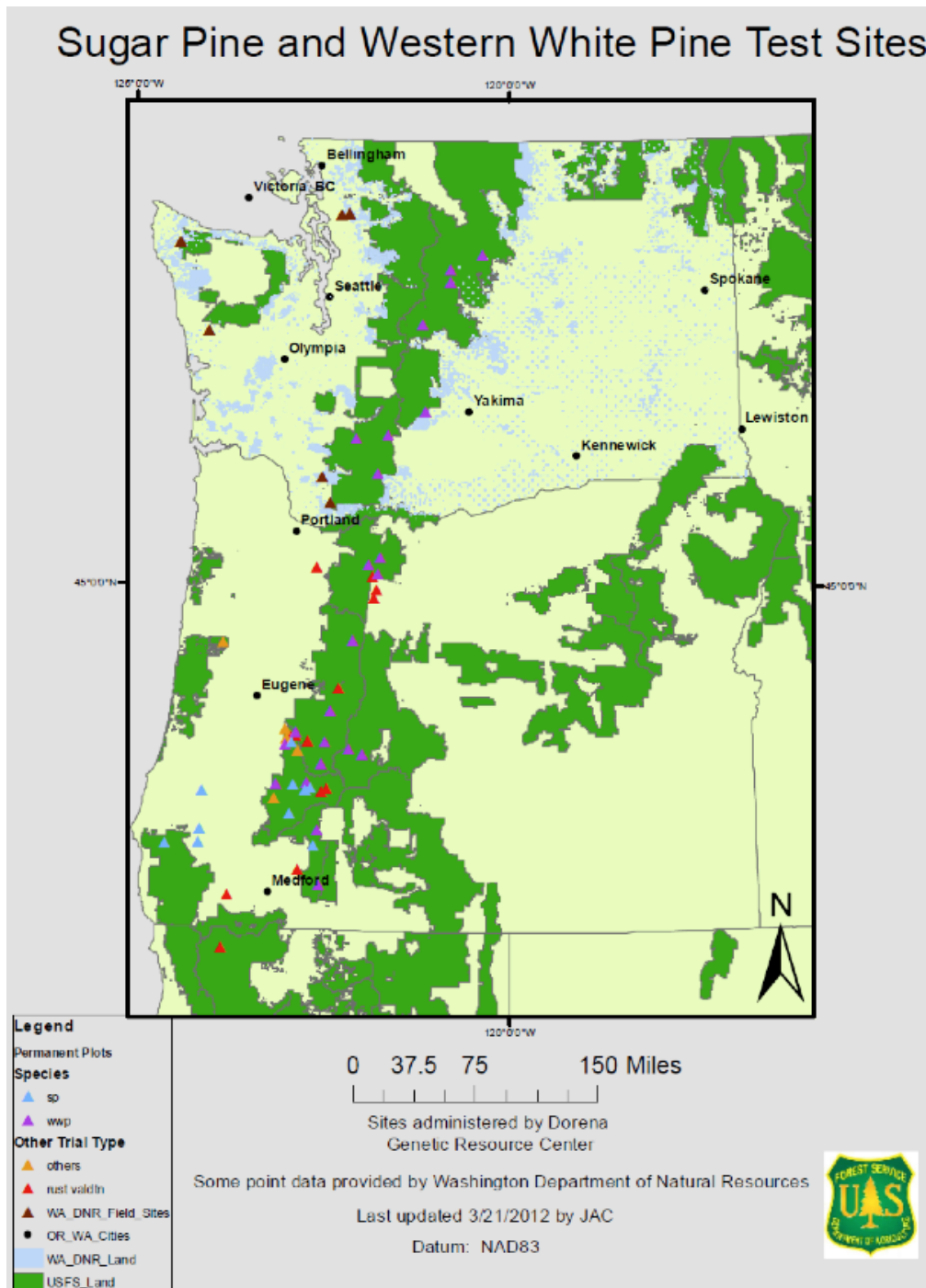


Figure 7. Locations of western white pine and sugar pine blister rust resistance field trials in Region 6.

The number of stem infections per tree can vary five- to ten-fold among families (Kolpak and others 2008; Sniezko, unpublished data), and individual trees can have hundreds of stem infections (Figure 5). It is not surprising that families with partial resistance show moderate to high level of infection at sites with a high rust hazard, they generally performed similarly in the artificial inoculation trials (Figures 10 & 11). However,

mortality often lags several to many years behind infection and for many of these families we need more time to examine what percentage of trees will remain canker-free as well as what percentage of trees with stem infections will survive. In a field trial that included families with a wide array of resistance based on earlier seedling screening, the relationship between results from seedling screening using artificial inoculation and

field resistance is encouraging (Figures 10 & 11; also Kolpak and others 2008). The level of field resistance in these first families tested is encouraging, and the general leveling-off of infection suggests the resistance may be durable. However, more time is needed to examine the surviving trees under changing rust hazards due to climate change and as well as to continue to evaluate any potential evolution toward increasing pathogenicity in the rust fungus. Ultimately, success in reforestation and restoration plantings using orchard seed will further determine the durability of resistance (for some recent surveys of operational reforestation using resistant seed, in Idaho, see Kearns and others 2012).

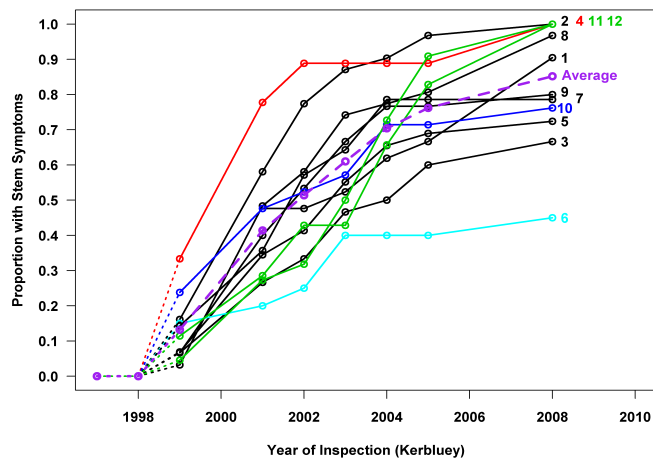


Figure 8. Time trend for 12 western white pine families for 1997 Kerbluey planting for proportion of trees with stem symptoms from blister rust infection (first assessment in 1999 Rust infection (percentage of trees with stem symptoms) of 12 western white pine families at Kerbluey site on Cottage Grove Ranger District, Umpqua NF. Nine families (wild OP) with partial resistance, one susceptible control (wild OP, #4), and two families (#11 & 12) with major gene resistance shown (figure adapted from Sniezko and others 2012b).

SEED ORCHARDS

Resistant selections are grafted and placed into seed orchards. Orchards for several breeding zones are now producing seed in Region 6. The selection intensity used for the parents in the initial orchards varies, but is relatively low, to establish the genetic base large enough for future breeding. In general, the parents used to establish the first cycle of western white pine orchards are within-family selections from the seedlings in the artificial inoculation trials. In some orchards both

complete and partial resistance is included, in other orchards only partial resistance is included. The first available orchard lots have been included in recent field trials, and results are pending. This first set of orchards is expected to offer modest gains in rust resistance but sets the stage (as seed cone and pollen production start) for breeding to increase the levels and diversity of resistances. Breeding to increase the level of resistance and combine resistances is now underway, but this will need to be continued for a number of years to develop the higher levels of resistance and to provide the next generation of seed orchards. Resistance testing of the first of these advanced-generation seedlots is also underway (Figure 6).

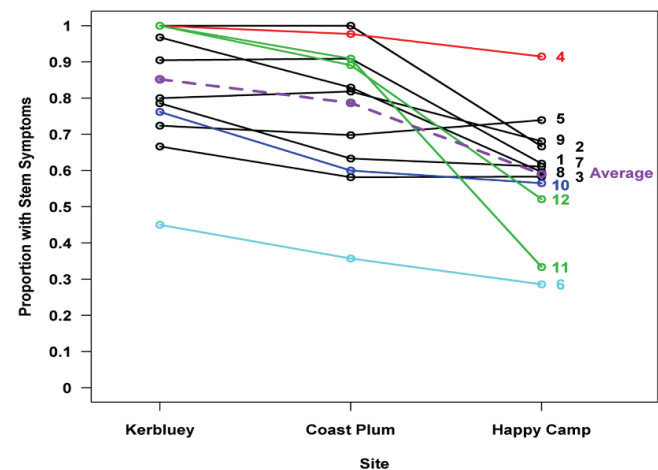


Figure 9. Family means at last assessment at three sites for 12 families for proportion of trees with stem symptoms. Nine families (wild OP) with partial resistance, one susceptible control (wild OP, #4), and two families (#11 & 12) with major gene resistance shown (figure adapted from Sniezko and others 2012b).

THE RUST FUNGUS AND EVOLUTION OF VIRULENCE & INCREASED AGGRESSIVENESS

The rust fungus is genetically variable, but more information is needed on its evolutionary potential and how this might affect resistance in western white pine. Virulence against the Cr2 resistance gene is already well documented (Kinloch and others 2004; Kolpak and others 2008; Sniezko and others 2012b; <http://www.fhm.fs.fed.us/posters/posters01/geo.pdf>), and from seedling trials some evidence also exists of increased aggressiveness in some rust populations (Hoff and McDonald 1993; McDonald and others 1984; Sniezko, unpublished). However, more information is needed on

which partial resistance mechanisms may be impacted and the extent of any erosion of resistance.

The Eurasian white pine species are generally much more resistant to blister rust (Hoff and others 1980; Sniezko and others 2008), presumably because they have co-evolved with the pathogen. However, as recent summaries indicate, even these species can be negatively impacted by blister rust (La 2008; Zhang and others 2010). There are likely two species of white pine blister rust in China (Zhang and others 2010), including *C. ribicola*. These Eurasian sources of rust likely harbor different genes for pathogenicity and could further threaten North American native species of white pines if they were accidentally introduced here. More knowledge on the co-evolved pathosystem of the rust and white pines of Asia and its breakdown in some areas is needed. Joint resistance testing with colleagues in Asia or Russia would also be very beneficial.

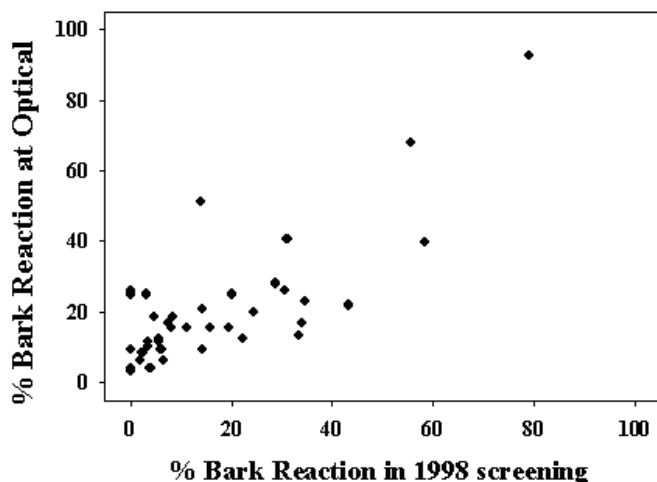


Figure 10. Relationship between percent bark reaction at Optical field site and in seedling screening at Dorena GRC for 38 families. Data summarized through six years exposure to rust at Optical and five years after inoculation at DGRC. Pearson correlation $r = 0.76$, $p < 0.001$ (Figure from Kolpak and others 2008).

SUMMARY

The first cycle of selection and rust resistance testing was used to establish a wide genetic base for breeding and the first seed orchards for the breeding zones in the Pacific Northwest Region. The thousands of field selections made vary dramatically in the type and level of their rust resistance, but as expected only a few rare first-generation selections of western white pine offer moderate levels of resistance to the non-native blister

rust pathogen. Selections from within families in the artificial inoculation trials were used to establish the initial round of seed orchards which are producing the first available resistant seed and provide the new parents for breeding for increase resistance.

The complete resistance (HR) available from Cr2 gene provides a high level of resistance, but is very vulnerable to virulent *vcr2* genotypes of the rust which is now found in many areas of western Oregon. Partial resistance will be the key to durability of rust resistance and is the focus of ongoing breeding and rust testing. Establishment of advance-generation orchards will continue to raise the level of rust resistance, increasing their utility to land managers. Both complete and partial resistance will be incorporated into some seed orchards.

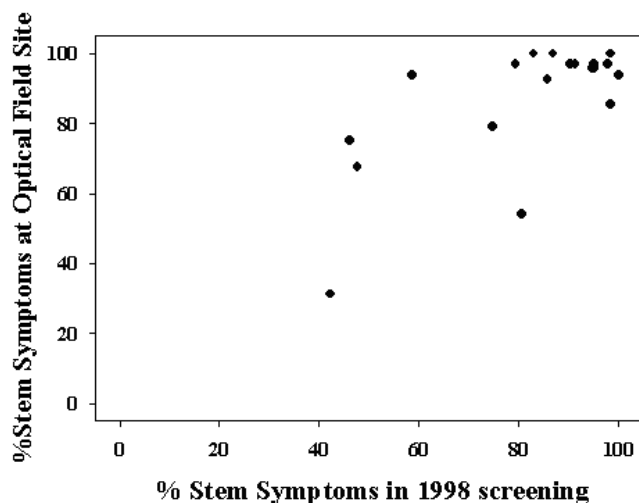


Figure 11. Relationship between percent stem symptoms at Optical field site and seedling screening at Dorena GRC for 20 non-Cr2 families. Data summarized through six years exposure to rust at Optical and five years after inoculation at DGRC. Pearson correlation $r = 0.71$, $p < 0.001$. (Figure from Kolpak and others 2008).

The infrastructure in place, the expertise available, and the protocols established over decades of work at Dorena Genetic Resource Center have greatly facilitated the screening of other species of white pines for resistance to white pine blister rust, as well as permitting the rapid implementation of an operational program to develop resistance to *Phytophthora lateralis* in Port-Orford-cedar (Sniezko and others 2012e). In less than a decade, hundreds of seedlots of the other white pine species such as whitebark pine and limber pine have been evaluated for resistance and relatively high levels and frequency of resistance have been found

(Schoettle, unpublished; Sniezko and others 2011; Sniezko, unpublished). The level of genetic resistance in some of these species, such as whitebark pine (at least in the Pacific Northwest populations), appears to be higher than that found in most of the sugar pine or western white pine field selections tested. The ongoing operational programs in Region 6, as well as those in Regions 1 and 5, and British Columbia, provide one of the first examples of using genetic resistance in native conifers to help ensure that a species retains its utility in managed forests or maintains a place in natural forest ecosystems in the face of invasive by a non-native pathogen (Sniezko 2006; 2012b).

Continued monitoring of current field trials is needed to determine which resistances are most effective across which sites and the durability of the resistances in the face of a potentially evolving rust pathogen. Control pollinations among progeny selections (and their rust testing) and establishment of advanced-generation orchards will be needed to increase the level and mix of resistances available for operational use. These future orchards will be the key to fully optimizing the potential to retain species such as western white pine and sugar pine in our forest ecosystems or managed plantations.

Most of the families included in current field trials are open-pollinated seedlots from some of the best original selections from the forests, or crosses among some of these parents. However, more within-family selections made in rust screening trials are starting to produce seed cones and pollen and crosses among them are underway, and a few are in testing. It will be essential to capitalize on this initial improvement in resistance to increase resistance further.

This development of populations of forest tree species with genetic resistance utilizes the naturally occurring genetic variation within these species to restore ecosystems or retain a species for utilization in reforestation. Developing and utilizing genetic resistance is one of the few management options available with the potential to successfully restore or retain these species, and unlike other options it is the path with few, if any, negative environmental consequences. By their nature, these operational programs to develop genetic resistance to blister rust are long-term, multi-generation endeavors that seek to correct the imbalance brought forth by introduction of a non-native invasive pathogen that has disrupted the

natural balance that existed. Due to the long-term nature of these programs their success depends on strong, continued management support as well as the required facilities and the team of scientists, technicians and land managers working together to accomplish the goal of developing and utilizing resistant populations of these species. The Forest Service is a world leader in developing and utilizing genetic resistance.

ACKNOWLEDGMENTS

The support of the Genetic Resource and Forest Health Protection programs has been a key to the progress to date in the resistance program. Our cooperators and partners such as BLM and numerous other land managers have also provided critical resources for advancing the resistance program. The Dorena GRC employees and their counterparts on the many National Forests have also made invaluable contributions. Doug Savin provided several of the graphs and Jennifer Christie provided the maps.

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FROM DIAGNOSTICS TO METAGENOMICS: APPLICATIONS OF DNA-BASED TOOLS IN FOREST PATHOLOGY

Amy L. Ross-Davis^{1,2}, Mee-Sook Kim³, Jane E. Stewart⁴, John W. Hanna¹, John D. Shaw⁵, and Ned B. Klopfenstein¹

INTRODUCTION

Advances in molecular technology provide an accessible set of tools to 1) help forest pathologists detect, identify, and monitor forest pathogens, 2) examine the evolutionary relationships and global distributions of forest pathogens and their hosts, 3) assess the diversity and structure of host and pathogen populations, and 4) evaluate the structure and function of genes, as well as their levels of expression, within species and within communities. This paper briefly outlines DNA-based tools, including molecular diagnostics, phylogenetics, population genetics, genomics, and metagenomics, and discusses how each has been or could be applied in the field of forest pathology.

MOLECULAR DIAGNOSTICS

DNA-based diagnostics use DNA-sequence data to identify or characterize biological organisms (e.g., Hoff et al. 2004, Glaeser and Lindner 2010). To help identify organisms, DNA-based diagnostics typically rely on comparisons with a worldwide database, such as GenBank[®] (<http://www.ncbi.nlm.nih.gov/genbank/>) that contains DNA sequences from known species. The utility of DNA-based diagnostics continues to increase, and DNA databases continue to expand. Morphology still plays a role in the identification of fungal species; however, members of different species can have similar morphology, and members of the same species can have very different morphology. Similarly, mating tests still play a role in the identification of fungi based on the biological species concept, but very different fungal species are sometimes inter-fertile. Thus, fungal identification continues to become more reliant on DNA-based methods. In forest pathology, DNA-based

diagnostics are frequently used to detect, identify, and monitor forest pathogens and other microbes, largely because these methods are reliable, relatively quick, and economical.

Fusarium root disease in forest nurseries is one of the best examples of the utility of DNA-based diagnostics. *Fusarium oxysporum* is morphologically indistinguishable from *F. commune* (Stewart et al. 2006). *Fusarium commune* is an aggressive pathogen that causes damping off of Douglas-fir (*Pseudotsuga menziesii*) seedlings, whereas *F. oxysporum* is apparently non-pathogenic on Douglas-fir seedlings (Stewart et al. 2006, 2012). Furthermore, a recent paper by Dumroese et al. (2012) shows that some *F. oxysporum* isolates have biological control potential to protect seedlings from damping off disease caused by *F. commune*. DNA-based diagnostics is the only available method to distinguish these two fungal species.

Another example of the utility of DNA-based diagnostics lies with *Armillaria*. The pathogenic species *A. solidipes* [pending vote to conserve *A. ostoyae* (Redhead et al. 2011)] is often difficult to distinguish from the other, saprophytic species in the genus because it is typically observed in a vegetative state. Since DNA-based methods have become available to discriminate among North American *Armillaria* species (Kim et al. 2006, Ross-Davis et al. 2012a), these approaches have been used to detect the occurrence of *A. solidipes* throughout much of the west as part of a collaborative effort with USDA Forest Service, Forest Health Protection and Forest Inventory and Analysis (FIA) to predict where *A. solidipes* is likely to occur and cause disease under different forest management regimes and predicted conditions associated with a changing climate (e.g., Hanna et al. 2009, McDonald et al. 2011, Klopfenstein et al. 2012).

Similarly, DNA-based diagnostics were essential to determine that the white pine blister rust pathogen (*Cronartium ribicola*) could use non-*Ribes* alternate hosts, such as *Pedicularis* sp. and *Castilleja* spp., to

In: Browning, J. Comp. Proceedings of the 60th Annual Western International Forest Disease Work Conference; 2012 October 8-12; Tahoe City, CA.¹USDA Forest Service-RMRS, Moscow, ID. ²Western Forest Conservation Association. ³Kookmin University, Seoul, South Korea. ⁴USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR. ⁵USDA Forest Service-RMRS, Ogden, UT.

complete its lifecycle (e.g., McDonald et al. 2006). This phenomenon may have been overlooked previously because infections on these non-*Ribes* hosts were perhaps attributed to *C. coleosporioides*, the cause of stalactiform rust of “hard” pines. It remains undetermined whether the use of non-*Ribes* alternate hosts by *C. ribicola* is a partial explanation for the shortcomings of the 50-year *Ribes* eradication effort to control white pine blister rust.

The utility of DNA-based diagnostics continues to grow in forest pathology. These techniques provide reliable identification of native and exotic pathogens, mycorrhizal fungi, symbionts, biocontrol agents, endophytes, decay fungi, and other microbes associated with forest ecosystems (e.g., Hoff et al. 2004, Glaeser and Lindner 2010). The accurate identification of forest microbes is essential to better understand their distribution and ecological relationships within forest ecosystems.

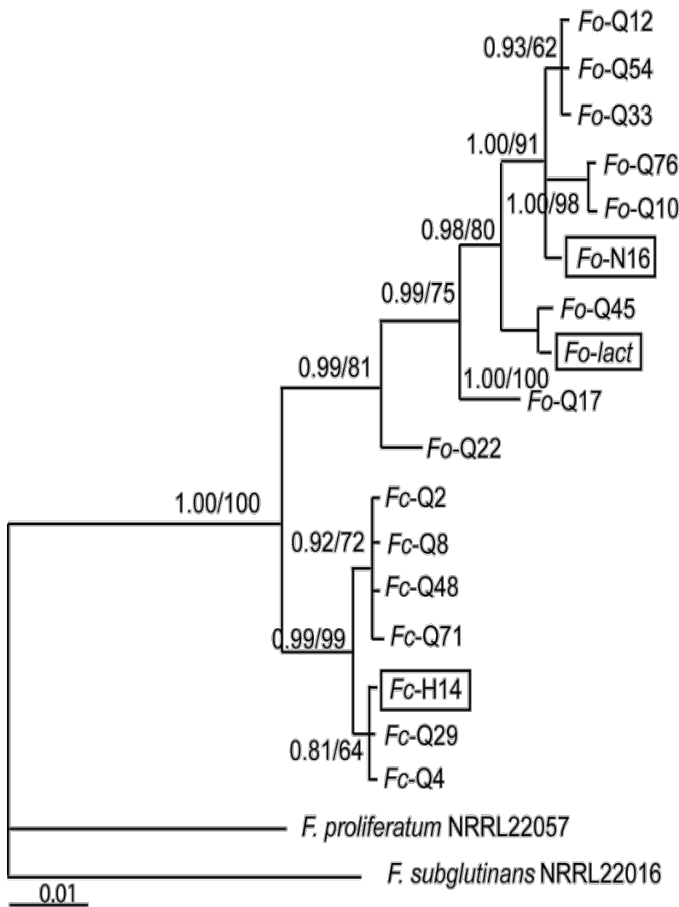


Figure 1. Phylogenetic relationship between the non-pathogenic *Fusarium oxysporum* (*Fo*-) and the highly virulent *Fusarium commune* (*Fc*-) (from: Stewart et al. 2012).

PHYLOGENETICS

Phylogenetics is the study of evolutionary relationships among groups of organisms, and phylogenetic analysis is often based on DNA-sequence data. Our definitions of fungal species are becoming increasingly reliant on the phylogenetic species concept (Taylor et al. 2000), because it allows detection of species at a finer scale than most other speciation concepts and it seems to parallel the ecological species concept (Andersson 1990). As shown in Figure 1 (based on Stewart et al. 2012), phylogenetic analysis was able to determine a distinct genetic difference between the non-pathogenic *F. oxysporum* and the highly virulent *F. commune*, which fall into separate genetic groups when morphologically these two species are indistinguishable.

The genus *Armillaria* is another example where phylogenetics can contribute to our understanding of species delineations. *Armillaria* species have great variation in distribution and ecological behavior, with some species possessing the potential to be invasive pathogens. Each species may vary in their habitat, host range, and response to climate change. Morphology-based methods and compatibility-based methods have contributed greatly to the identification of *Armillaria* species, but phylogenetic methods are proving to be more precise. Currently, phylogenetic analysis based on the translation elongation factor-1 α (*tef-1 α*) gene sequences provides the best available method for distinguishing North American *Armillaria* species. Figure 2 depicts a phylogenetic tree of North American *Armillaria* species, based on DNA sequences from the *tef-1 α* (Ross-Davis et al. 2012a), and efforts are now underway to examine the utility of this method for delimiting diverse *Armillaria* species from across the Northern Hemisphere (see Klopfenstein et al. this proceedings).

The aforementioned studies and many others clearly demonstrate that phylogenetic studies are essential to better understand genetic and evolutionary relationships among forest microbes and forest hosts. The application of more powerful phylogenetic tools will contribute to improved definitions of host and microbe species. Furthermore, phylogenetic tools are useful for determining sources of invasive pathogens or predicting sources of potentially invasive pathogens before they are introduced.

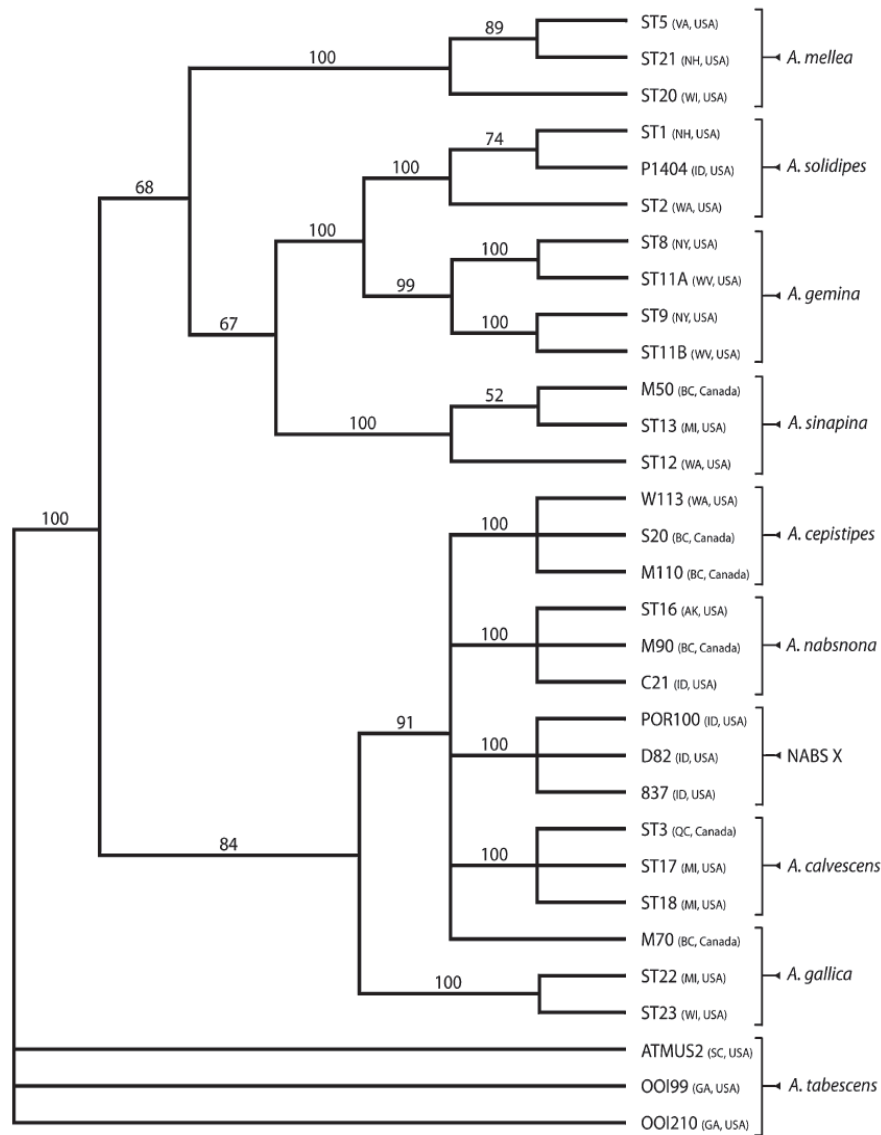


Figure 2. Phylogenetic relationship among North American *Armillaria* species (from: Ross-Davis et al. 2012a).

POPULATION GENETICS

In addition to determining species and their relationships, defining the relationships of pathogen population structure in association with host, environment, and geography allows a more precise examination of the biological processes of forest disease. Population genetics allows us to examine genetic relationships among populations by studying allele frequency distribution and change under the influence of four main evolutionary forces (natural selection, genetic drift, mutation, and gene flow and transfer). Several previous studies have examined the population structure of pathogens. For example, Hamelin et al. (2000) found distinct *Cronartium*

ribicola populations in eastern and western North America, and Richardson et al. (2008) found that major gene resistance appears to have imposed strong selection pressure on the blister rust pathogen. Winton et al. (2006) identified two lineages of Swiss needle cast pathogen (*Phaeocryptopus gaeumannii*) in Oregon and Washington, where only one lineage was associated with disease epidemics. Zhang and Blackwell (2002) reported that the dogwood anthracnose pathogen (*Discula destructiva*) exists as distinct eastern and western populations. In addition, Baumgartner et al. (2010) demonstrated the existence of two geographically isolated, divergent genetic pools of *A. mellea* in the USA.

In addition to pathogen species, we can also examine the population structure of host species, which is important for delimiting seed transfer zones and focusing conservation efforts. Kim et al. (2011) showed that southern western white pine (*Pinus monticola*) populations (below 45°N latitude) are more genetically diverse than northern populations, but most of this genetic variation is attributable to differences among individuals within populations rather than differences among populations. This is not surprising given that this is a wind-pollinated species so gene flow is unobstructed. The definition of western white pine populations is essential for species management and conservation programs that are addressing the threats of white pine blister rust, climate change, and other environmental threats.

Aspen (*Populus tremuloides*) is the most widely distributed tree species in North America and one that is currently threatened by a sudden aspen decline (Worrall et al. 2010). Mock et al. (2008) used genetic markers to determine genetic diversity and clonal structure of aspen stands. In a subsequent continental-scale study of aspen, Mock et al. (2012) found that triploidy was common in the central Rocky Mountains and rare in almost all other parts of aspen's range. The proportion of triploidy in local populations was highest in areas characterized by relatively high summer temperature and low precipitation (Figure 3). Although sudden aspen decline has been associated with climate stress (Rehfeldt et al. 2009, Worrall et al. 2010), the resistance/susceptibility of triploid aspen remains unknown.

Population genetics allows us to define units of hosts and pathogens that may respond similarly to environmental threats, identify valuable and unique populations for species management and restoration, make predictions of potential effects of environmental threats, and identify mechanisms behind invasions by exotic forest pathogens. Furthermore, understanding population structure of forest trees and pathogens is essential for efforts to restore, conserve, and sustain forest tree species during an era of climate change.

THE “-OMICS”

Genomics is the study of the structure and function of genes. A genome is the entirety of an organism's hereditary information (both coding and non-coding). Much progress has been made since the mid-1970s when the first complete nucleotide sequence of a viral RNA-genome was established (Fiers et al. 1976) and then the first DNA-based genome was sequenced (Sanger et al. 1977). In the mid-1990s the first bacterial genome was sequenced (Fleischmann et al. 1995) followed by the first eukaryotic genome of the budding yeast (<http://www.yeastgenome.org/>). In 2003 the 13-year effort to map and sequence the human genome was completed (International Human Genome Sequencing Consortium 2004). When accessed in October 2012, the genome online database (<http://www.genomesonline.org/cgi-bin/GOLD/index.cgi>) listed 18,378 genome projects, of which 3,762 had been completed. At the same time, the Department of Energy (DOE) Joint Genome Institute (JGI) Fungal Genome Program (<http://genome.jgi.doe.gov/programs/fungi/index.jsf>) listed 744 fungal genome projects, many of which had been completed, including several species of white rot fungi, brown rot fungi, and other pathogenic species. The 1000 Fungal Genomes Project (<http://1000.fungalgenomes.org/home/>) is a 5-year collaborative project to sequence 1000 fungal genomes across families allowing for the comparative genomics of fungal pathogens to look for common pathogenicity mechanism strategies and new ways to combat pathogens.

Transcriptomics is also known as expression genomics and is the study of that portion of the genome that is transcribed into mRNA molecules at any given time and in a given environment. It allows us to study gene expression in different tissues, at different time points, and under different conditions. By comparing how gene expression differs we can begin to identify genes associated with certain functions. For example, more than 1000 genes in hybrid poplar are up-regulated in response to insect attack as reported by Ralph et al. (2006). In the *Populus-Melampsora* pathosystem, differential regulation of genes involved in pathogenesis have been revealed via transcript profiling in susceptible versus incompatible interactions (Rinaldi et al. 2007), susceptible interactions over time (Miranda

et al. 2007) and susceptible interactions as infected with two different *Melampsora* species (Azaiez et al. 2009).

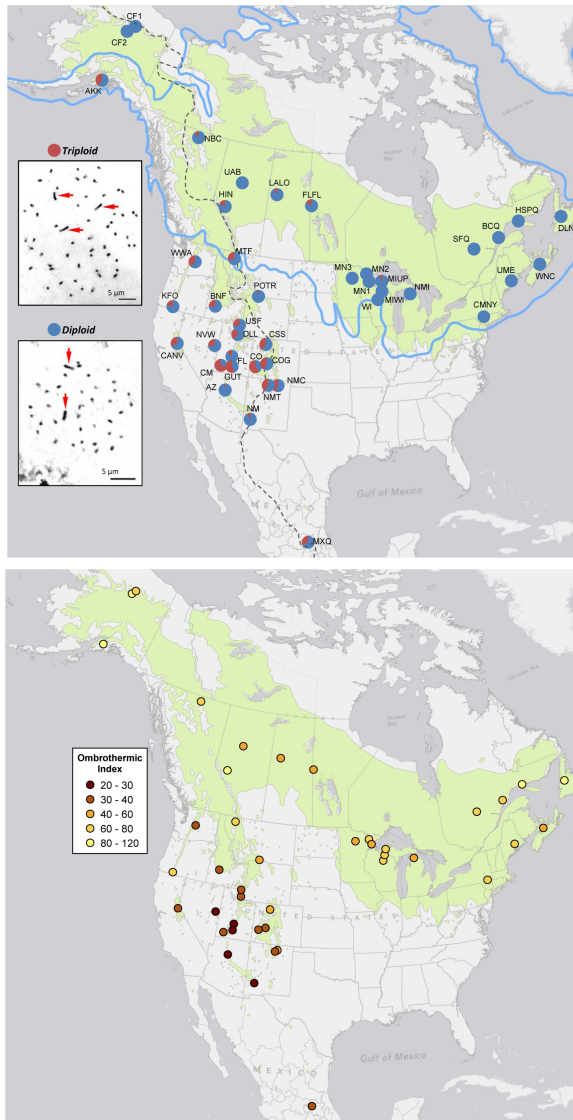


Figure 3. Top. Map of diploid:triploid proportions for 42 local populations of aspen in North America. **Bottom.** Ombrothermic index for aspen population centroids (low = warm-dry summer, high = cool-wet summer). (from: Mock et al. 2012).

CASE STUDY

We embarked on a study to characterize a transcriptome of a mycelial fan of a widespread, virulent clone of *A. solidipes* (isolate RNA1), and focus particularly on the identification of genes involved in degrading plant cell wall components and responding to the host environment (Ross-Davis et al. 2012b). RNA was stabilized, isolated, and then sequenced to generate over 24 million short reads which were then assembled *de*

novo (since a reference genome for *Armillaria* does not yet exist) to generate almost 40,000 contigs – or consensus regions of DNA. We then compared these contigs to genes in the NCBI database and found almost 20,000 significant alignments, over 12,000 of which have known functions. One concern with *in planta* transcriptomics is isolating the pathogen RNA from the host tissue. Of the significant alignments, most contigs aligned best to fungal sequences, mostly within the phylum Basidiomycota, and the order Agaricales into which *A. solidipes* is classified. Only 54 of the alignments were to sequences from *Armillaria* species due to the limited availability of DNA sequences from this taxon in the database. The ability to use the host as a carbon source plays an essential role in pathogenesis. White rot fungi like *A. solidipes* are capable of efficiently degrading all components of plant cell walls including cellulose, hemicellulose, pectin, and lignin. In characterizing this transcriptome, we found evidence for the use of an array of enzymes involved in plant cell wall degradation (Table 1).

Metagenomics is also known as environmental genomics, ecogenomics, community genomics, and megagenomics and is the study of genetic material recovered directly from environmental samples. Microbial genome sequencing traditionally relied on clonal cultures but early environmental gene sequencing, which cloned specific genes to produce a profile of diversity in a natural sample, revealed that most of the biodiversity had been missed by these culture-based methods. This is not surprising given the estimate that a single gram of soil contains billions of microbial cells, only a fraction of which can be grown in culture. The term metagenomics first appeared in publication in 1998 (Handelsman et al. 1998) and has been used to examine the microbiome of marine environments, beginning with Craig Venter’s work almost a decade ago (Venter et al. 2004) and now with 179 marine metagenomics published papers catalogued in the Thomson Reuters Web of KnowledgeSM. Much work is also being done on human systems. For example, the Human Microbiome Project (<http://commonfund.nih.gov/Hmp/>) seeks to describe the diversity of microbial species associated with health and disease. Since metagenomics allows us to tap into the metabolic potential of uncultivated microbes we can now discover novel genes and novel metabolic pathways with clear applications for biotechnology,

biofuel production, and bioremediation. Most applicable to the field of forest pathology is the application of metagenomic approaches to understanding soil microbial communities and how these relate to disease suppression (van Elsas et al. 2008a and b, Hernandez-Leon et al. 2010, Mendes et al. 2011, Klein et al. 2013). This is a very active area of research with over 200 papers currently published in the area of soil metagenomics as catalogued in the Thomson Reuters Web of KnowledgeSM.

Essentially, the three general steps involved in a metagenomics project are (1) sequencing, (2) processing - which includes (a) assembling the multitude of sequencing reads, (b) annotating these assembled contigs to assign function, and (c) assigning contigs to specific taxa, and then finally (3) analysis - which refers to examining the functional and metabolic diversity of microbial communities. One challenge associated with metagenomics is the generation and analysis of massive amounts of sequence data. While the efficiency associated with high-throughput sequencing has changed metagenomics so that sequencing DNA is no longer the limiting factor, a bottleneck now lies in the area of computational

analysis and interpretation. Nonetheless, international collaborations for sharing information and coordinating sequencing and bioinformatics activities, such as terragenome (<http://www.terragenome.org/>), exist so that these efforts can be met with success.

Disease-suppressive soils are exceptional ecosystems in which crop plants suffer less from specific soil-borne pathogens than expected because of the activities of other soil microorganisms. Metagenomics of the rhizosphere microbiome coupled with culture-dependent functional analyses identified key bacterial taxa and genes involved in suppression of a fungal root pathogen (e.g., Mendes et al. 2011, Klein et al. 2013). Such studies show that specific constituents of the microbial community help protect plants against pathogen infections. Similar approaches in forest ecosystems can begin to uncover the role of the soil microbial community, particularly certain taxa and genes, in suppressing various forest diseases. And, by linking metadata (or specific environmental factors like fire, thinning, climate) to the presence of beneficial or harmful taxa and to the expression of specific genes, we can begin to identify management practices that can effectively address root disease.

Table 1. Enzymes involved in plant cell wall degradation expressed in *Armillaria solidipes* isolate, RNA1.

Enzymes	Orthologs
<i>Ligninolytic and related enzymes</i>	
Laccase (EC 1.10.3.2)	10
Manganese peroxidase (EC 1.11.1.13)	5
Versatile peroxidase (EC 1.11.1.16)	4
Aryl alcohol oxidase (EC 1.1.3.7)	4
Alcohol oxidase (EC 1.1.3.13)	1
(S)-2-hydroxy-acid oxidase (EC 1.1.3.15)	1
D-arabinono-1,4-lactone oxidase (EC 1.1.3.37)	4
Cytochrome P450s	37
<i>Cellulolytic, hemicellulolytic, and related enzymes</i>	
Cellulase (EC 3.2.1.4)	9
β -glucosidase (EC 3.2.1.21)	3
Cellulose 1,4- β -cellobiosidase (non-reducing end) (EC 3.2.1.91)	8
Feruloyl esterase (EC 3.1.1.73)	1
β -galactosidase (EC 3.2.1.23)	18
α -mannosidase (EC 3.2.1.24)	7
Mannan endo-1,4- β -mannosidase (EC 3.2.1.78)	4
Endo-1,4- β -xylanase (EC 3.2.1.8)	4
Xylan 1,4- β -xylosidase (EC 3.2.1.37)	1
α -N-arabinofuranosidase (EC 3.2.1.55)	3
Xylan α -1,2-glucuronosidase (EC 3.2.1.131)	2
xyloglucan-specific exo- β -1,4-glucanase (EC 3.2.1.155)	1
Cellobiose dehydrogenase (EC 1.1.99.18)	1
<i>Pectinolytic enzymes</i>	
Pectinesterase (EC 3.1.1.11)	2
Feruloyl esterase (EC 3.1.1.73)	1
Polygalacturonase (EC 3.2.1.15)	13
Pectate lyase (EC 4.2.2.2)	9

DNA-BASED ANALYSES AND STRATEGIC FOREST INVENTORIES

The availability of environmental data is essential to provide ecological interpretations for genetic analysis of forest pathosystems at diverse spatial scales. Even as methods for DNA-based analyses continue to progress for application in forest ecosystems, meaningful ecological insights are dependent on obtaining geographically distributed, representative samples with accompanying environmental data. Even in well-studied areas like western North America, the distributions of some pathogens remain only partially mapped.

The USDA Forest Service FIA program samples a wide variety of forest characteristics using a sampling grid with approximately 5-km spacing in all U.S. States and Territories (approximately 135,000 forested plots nationwide). Individual plots are remeasured on a 5- to 10-year cycle, depending on the state. By linking DNA-based analyses to such an inventory system, the presence, absence, and effects of pathogens may be linked to environmental variables and associated stand/site characteristics. Furthermore, responses of forest pathogens to any changes in the environment, whether through successional processes, common disturbance events (e.g., insect infestations or fire), or long-term climatic change may be monitored during successive inventory cycles. The combination of high-resolution genetic data with extensive environmental metadata, offers great potential to understand forest ecosystem processes, including forest diseases, at an unprecedented level. However, methods must be developed to allow the efficient collection and analyses of genetic data from well-characterized sites. Once field and laboratory methods can be scaled up for application in a production inventory environment, the potential for understanding genetic and environmental interactions, as they relate to forest disease, will increase substantially. Thus, the availability of a permanent plot network with associated environmental data, such as those of FIA, provide an invaluable opportunity to derive increased ecological information from the application of DNA-based tools to better understand interactions among forest pathogens, hosts, other components of the abiotic and biotic environment, and stand history.

CONCLUSION

In summary, we can (1) detect, identify, and monitor forest pathogens and other organisms through molecular diagnostics, (2) determine the evolutionary relationships and global distributions of forest pathogens and their hosts through phylogenetics, (3) assess the diversity and structure of host and pathogen populations through population genetics, and (4) evaluate the structure and function of genes within species and communities with genomics and metagenomics. Although, the power and utility of these genetic tools continues to grow at a very fast pace, their utility is also dependent on the availability of environmental metadata at diverse spatial scales.

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DEVELOPMENT OF A DNA SAMPLING KIT TO DETECT PATHOGENIC, SAPROTROPHIC, AND STAIN FUNGI IN SAPWOOD OF DECLINING RED PINE (*PINUS RESINOSA*) IN THE UPPER MIDWEST

M.T. Banik¹, D.L. Lindner¹, J. Juzwik², and J.A. Glaeser¹

ABSTRACT

An inexpensive kit was developed to collect wood samples for molecular detection of pathogenic, saprotrophic and stain fungi in declining *Pinus resinosa* in the Upper Midwest. The kit contained materials for “clean” collection of sapwood drill shavings, which were then subjected to PCR of the rDNA ITS region with fungal-specific primers, followed by cloning and sequencing. Twenty-seven stands with declining *P. resinosa* lacking obvious fungal fruiting bodies were sampled by natural resources personnel throughout MN, WI, and MI. No single mortality agent predominated and causal agent(s) likely differed by location. A complex interaction of abiotic and biotic agents is likely involved in the symptomatic stands. None of the selected trees tested positive for *Heterobasidion irregulare* with either a pathogen-specific primer or more general ITS primers. Root rot fungi that were detected in the declining and dead trees included *Armillaria solidipes* (= *A. ostoyae*), *Scytinostroma* sp., and two species of *Leptographium*, the genus associated with Red Pine Pocket Mortality. The brown rot fungi *Coniophora arida* and *C. puteana*, known to cause root and butt rots in conifers in the western U.S., were found on both living, declining trees and dead snags. Many ophiostomoid sapstain fungi were present and were usually associated with signs of insect activity. Saprotrophic decay fungi were prevalent with white rot fungi predominating, including *Amylostereum chailletii*, *Hyphoderma setigerum*, *Hypholoma fasciculare*, and *Trichaptum fuscoviolaceum*. Numerous ascomycetes, including endophytic fungi and yeasts, were recovered in great quantities; many of these were uncommon and site specific. Thirty-two unique sequences from basidiomycetes and 129 from ascomycetes had no species, genus, or family identification in GenBank, illustrating the complex and largely unstudied community of fungi found in declining *P. resinosa*. The procedures presented here can be used to address questions of fungal diversity and ecology as well as forest pathology, and the technique can be easily adapted to screening with species specific primers when screening for individual pathogens, thus reducing cost and labor. **Key words:** *Heterobasidion irregulare*, *Armillaria* Root Rot, Red Pine Pocket Mortality, ITS (internal transcribed spacer), root rot fungi, white rot fungi, brown rot fungi, sapstain, *Pinus resinosa*.

INTRODUCTION

Red pine, *Pinus resinosa* Ait., is the most widely planted tree species in the Lake States with nearly 1.9 million total acres in Michigan, Minnesota and Wisconsin (Gilmore and Palik 2006). Approximately 44 percent of the stands are on private land and 56 percent on public. Many plantings occurred in the 1930s and 1950s with most trees now in the pole and saw-timber size classes. The species is generally resistant to many insects and diseases and is typically unaffected by ice, snow or wind (Gilmore and Palik 2006).

In the past three decades, foresters and natural resource personnel in the Upper Midwest have observed unexplained thinning and discoloration of foliage in crowns of red pine and pockets of tree mortality in plantations. In Wisconsin, many of these symptoms are associated with a complex of insects and fungi termed “Red Pine Pocket Mortality (RPPM)” (Klepzig et al. 1991). The major biotic agents involved with this decline include the fungi *Leptographium terrebrantis* and *L. procerum* that are vectored by a variety of insects, including the red turpentine beetle, *Dendroctonus valens*, root collar weevil (*Hylobius radices*), pales weevil (*H. pales*), pitch-eating weevil (*Pachylobius picivorus*), and the bark beetle *Hylastes porculus* (Klepzig et al. 1991). The beetles feed on freshly cut stumps as well as the lower stems and roots of red pines, spreading the fungi from tree to tree. The fungi grow within the insect galleries and through root

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grafts to healthy trees, which are then stressed by decreased water conduction by the damaged roots. The stressed trees attract additional beetles and may ultimately be killed by the pine engraver beetle, *Ips pini*, and its fungal associate, *Ophiostoma ips* (Klepzig et al. 1991; Wisc. DNR 2011).

Other unexplained pockets of mortality have been attributed to Armillaria root disease, caused primarily by *Armillaria solidipes* (= *A. ostoyae*) (Kromroy 2004). In the eastern and upper midwestern U.S., this pathogen is usually associated with trees that are stressed by various biotic or abiotic factors, including drought, defoliation by insects or frost, foliage diseases, soil compaction, and flooding (Wargo and Shaw 1985). In some cases, the fungus acts as a primary pathogen, particularly in young conifer stands previously planted in areas dominated by hardwoods (Sinclair and Lyon 2005).

A third significant disease that causes crown fading and tree death is Heterobasidion root disease (HRD) caused by the fungus *Heterobasidion irregulare*. Prior to the early 1990s, HRD was not thought to be major disease problem in *P. resinosa* plantations of the Upper Midwest and was rarely reported in this region. In 1993, a fruiting body of *H. irregulare* was first collected by Dr. Glen Stanosz, University of Wisconsin – Madison, near Coloma, WI in Adams County on a *P. resinosa* stump with incipient root decay. Before this, records from the herbarium of the U.S. Forest Service Center for Forest Mycology Research (CFMR) in Madison, WI show that only two collections had been made in Minnesota and none in Wisconsin despite intensive collecting by noted mycologists H.H. Burdsall, Jr., Frances Lombard, M.J. Larsen and R. L. Gilbertson. The fungus had been observed more frequently in Michigan (J. O'Brien, personal communication; Stong and Lemmien 1964), including a collection by C.H. Kaufman from Houghton, MI that was placed in the University of Michigan Herbarium in 1908. By the end of 2011, HRD had been detected in 23 counties within Wisconsin (Scanlon 2011; Wisc. DNR 2011). Because species of *Heterobasidion* are considered endemic on susceptible hosts in the North Temperate Zone in North America, Europe and Asia, the pathogen may have been present at low levels on scattered coniferous and hardwood hosts in the region and not recognized until recently. The irregular and often infrequent fruiting of

the fungus may also help to explain the small number of historical detection reports in the Lake States. The increase in occurrence and severity of the disease over the past two decades may be attributable to the widespread planting of monocultures of red pine and subsequent thinning of those plantations. However, it is also possible that the pathogen is spreading into new regions of the Upper Midwest (Woodward et al. 1998).

Traditional diagnosis of HRD requires careful site inspection and laborious sampling methods. One common sampling technique involves excavation of two main roots on opposite sides of the suspect tree (Alexander and Skelly 1974). A short root segment is removed and submitted to a diagnostic laboratory for pathogen assay (e.g. incubation in a moist chamber and observation of the asexual reproductive structures of the fungus). In the past decade, drill shavings taken from trees and processed using molecular techniques have been used to identify fungi in forest stands and in urban settings (Guglielmo et al. 2010; Lindner et al. 2011). Thus, we proposed to modify such sampling protocols and laboratory methodologies to identify the fungi associated with unexplained crown decline and tree mortality in red pine stands in the Upper Midwest. Accurate diagnosis of a disease is necessary before appropriate preventive measures and control actions can be prescribed for such situations. The objectives of the study were: 1) to develop a protocol for use by state forest health specialists to sample red pines exhibiting unexplained crown thinning and discoloration, 2) to use primers (ITS1F/ITS4) that detect a broad range of fungal species to identify the fungi associated with declining trees, and 3) to use a primer specific for *Heterobasidion irregulare* to screen samples as a verification of the presence of this fungus in the region.

MATERIALS AND METHODS

Sampling Kit Composition and Distribution

Sampling kits were constructed for field personnel consisting of the following: fourteen sterile 1/8 in. diameter drill bits, fourteen 1.5 ml bottomless tubes ("microfunnels"), fourteen 1.5 or 2.0 ml sample collection tubes labeled with sample number, 1 larger tube of CTAB DNA extraction solution (Lindner et al. 2011), 3 disposable plastic transfer pipettes, 1 sample collection sheet with sample numbers matched to sample collection tube numbers, 3 sealed alcohol wipes,

1 small plastic bag for used items, and instructions on sampling procedure. Drill bits were sterilized by soaking in a 20 percent dilution (v/v) of 6 percent sodium hypochlorite bleach to remove all cross-contaminating DNA; all other plasticware and the extraction buffer were sterile. Each kit contained enough materials to sample a stand for two symptomatic trees, one control healthy tree, and an air sample control.



Figure 1. Sampling a freshly prepared wood surface after removing bark to cambium, taking care not to touch the exposed sampling surface.

Sampling Procedure

Field personnel were instructed to sample living, symptomatic trees that had no obvious fungal fruiting bodies. In each stand, two symptomatic trees were selected that had the general appearance of decline, including dieback and dead branches, but did not have fungal fruiting bodies. In some cases, stumps or snags were sampled rather than living trees. Symptomatic trees, stumps or snags were sampled at 4 points, representing the 4 quadrants of the lower stem, as recommended by Guglielmo et al. (2010). In addition, air samples and drill shavings from healthy trees were collected to serve as controls. These were collected before sampling of symptomatic trees to minimize the risk of cross contamination. Air sampling was conducted by holding two sample tubes open in the air for one minute; the tubes were agitated to ensure good airflow into the tube. Tubes were then capped and labeled as “air controls.” One “healthy,” non-symptomatic tree was chosen in each stand as an

additional control. Control trees were sampled at two points near the base of the tree using the same procedure as that described for symptomatic trees.

In each sampling, the blade of a hatchet or knife was initially wiped with 70 percent ethanol to prevent cross contamination from previous sampling. A “clean” surface on the stem of the tree was then prepared, about 6 in. above the soil surface (Figure 1) by using the knife or hand axe to remove the bark down to the cambium on a small section, taking care not to touch the exposed sampling surface. This area was then sampled using a sterile drill bit. Shavings were collected by placing the small end of a bottomless tube (“microfunnel”) against the clean exposed surface of the bole, with the long axis of tube parallel to the ground. The drill bit was placed through the microfunnel so that it rested against the sample point (Figure 2). Shavings were collected by drilling horizontally into the stem, “pumping” the drill in and out of the hole as the shavings collected in the microfunnel. When the microfunnel was full of shavings, the drill bit and tube were removed from the substrate, the drill bit being kept in the microfunnel to act as a plug. Shavings were then transferred to the sample collection tube by using the drill bit to force the shavings down the microfunnel into the tube (Figure 3). The procedure was repeated until approximately 0.5 ml of shavings were collected at the bottom of the collection tube. Sterile, new drill bits and fresh tubes were used for each sampling point. After leaving the field and in a cleaner environment (i.e. kitchen, laboratory, office, etc.), but within 4 hours of sample collection, the sterile transfer pipette was used to add enough CTAB DNA extraction buffer (Lindner et al. 2011) to the sample tubes to completely cover the shavings (approximately 1ml). Samples that were kept more than a few days before shipping were frozen. Thawed samples were sent to the Center for Forest Mycology Research in Madison, WI with collection information that included date, sample location, site number, collector, host, site information (including species composition, stand age/size class, soil type, etc.), and any observed symptoms.

Sampling Development and Verification

Using the techniques described above, three known positive trees with fruiting bodies of *H. irregulare* were sampled from a *P. resinosa* stand in Wild Rose, WI. Four samples were drilled at the base of the trees and

two samples were drilled along major roots exposed by digging. In one tree, 14 samples were collected at regular intervals along two roots exposed by excavation. Samples were assayed as described below.



Figure 2. Inserting the drill bit through the microfunnel before drilling into the sapwood.



Figure 3. Transferring the collected wood shavings from the microfunnel to the sample collection tube.

Sampling Locations

Forest stands were sampled by natural resources personnel in MI, MN, and WI. Site information is listed in Table 1 and shown in Figure 4. Collection information, including stand description, is presented in Table 1.

DNA Extraction, Cloning and Identification of Taxa

Samples received at CFMR were stored at -80°C until processed. For DNA extraction the 1.5 ml tubes containing the samples were thawed at 65°C for 1- 2 hours and then centrifuged at 16.1 rcf for 5 min at room temperature with 100 μL of the supernatant transferred to strip tubes. DNA was then extracted from samples using the technique of Lindner and Banik (2009) modified for use with 200 μL strip tubes as per Lorch et al. (2013). Amplification of the resulting DNA was accomplished using the fungal specific primer pair ITS1F/ITS4 following the technique of Lindner and Banik (2009) as was cloning of the resulting amplicons. Eight clones were chosen from each sample for reamplification and sequencing. Fungal identifications are based on the nearest BLAST match in GenBank using similarities of ≥ 97 percent to denote species identification and 90 – 97 percent for genus identification. Similarities of less than 90 percent were tentatively identified to higher level taxa that provided the best match (i.e., order or family). In addition, all samples underwent PCR with an *H. irregulare* specific primer (HA2, TACCCACGGCGTAGACA) paired with ITS1F. This primer was tested against the previously described diluted *H. irregulare* positive samples to verify its efficacy.

RESULTS AND DISCUSSION

A molecular testing kit was developed to sample declining *Pinus resinosa* trees for pathogenic, saprotrophic, and stain fungi using a “clean” drilling procedure followed by PCR, cloning and sequencing of the ITS region of DNA. The technique was refined using known positive trees. The methodology was initially tested on replicate samples taken at the base of the stem and along major roots of trees that had fruiting bodies of *H. irregulare* at their base. As all of these samples tested positive regardless of sampling position, a final protocol was developed in which four drill samples were taken from the base of the trunk in all 4 quadrants for suspected trees, following the procedure recommended by Guglielmo et al. (2010). The test kits were used to sample trees at 27 stands in MN, WI and MI during 2011. These were stands that appeared to be in a state of decline but in which no fungal fruiting bodies had been observed.

All 2011 Heterobasidion Sampled Sites

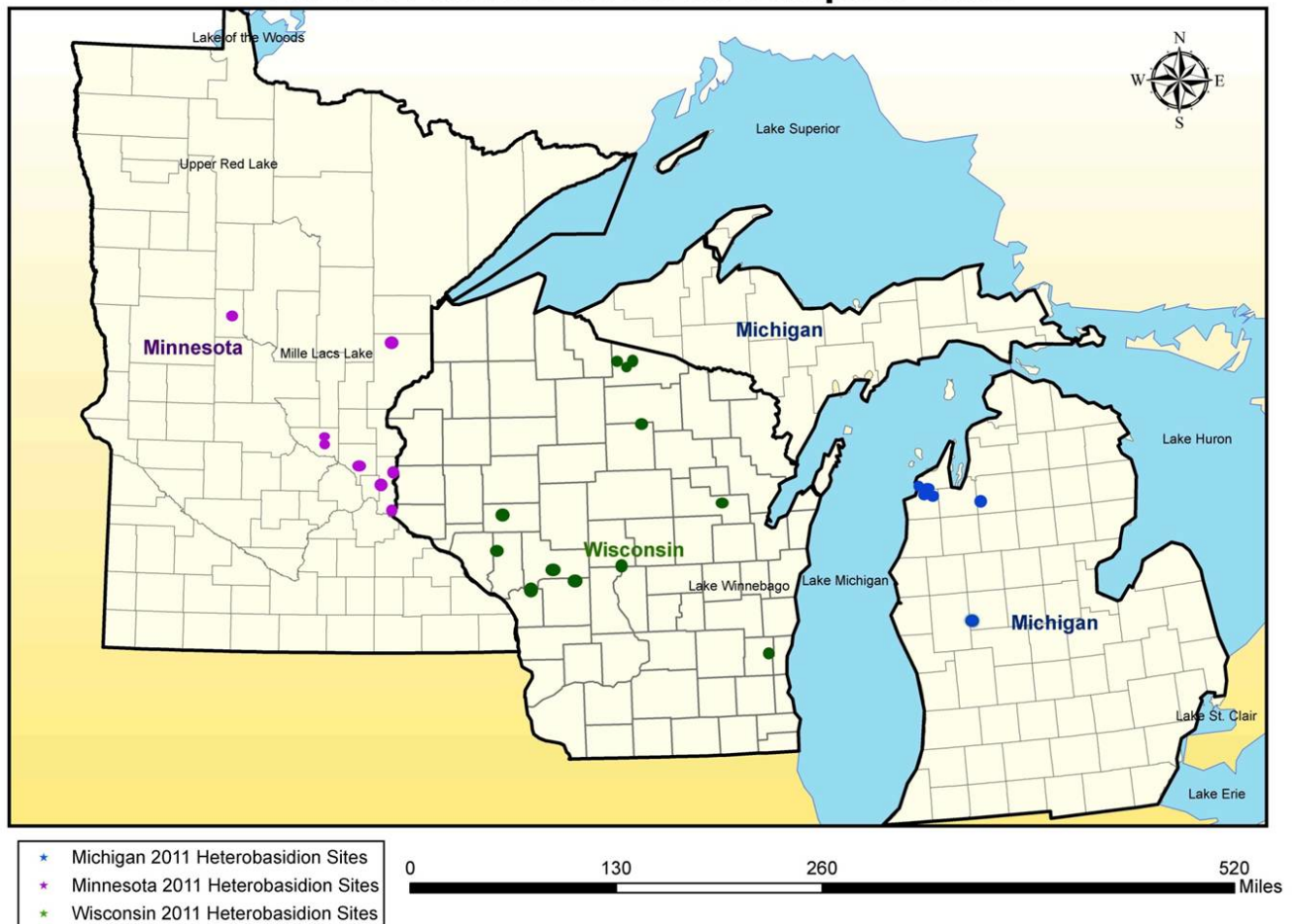


Figure 4. Location of sampling sites.

None of the selected trees tested positive for *Heterobasidion* either with the pathogen-specific primer or the more general, fungal-specific primers ITS1F/ITS4. The use of nonspecific primers made it possible to inventory the range of fungi present in declining trees (Table 2). Several other root rot fungi were detected in the declining and dead trees, including the white rot fungi *Armillaria solidipes* (= *A. ostoyae*) in Stands 14 and 26 and a species of *Scytinostroma* sp. – presumably *S. galactinum* - in Stands 22 and 25. The brown rotters, *Coniophora arida* (Stands 21, 22, 24) and *C. puteana* (Stands 17, 20), which cause brown cubical rot in the roots and bases of living conifers throughout the West (Rocky Mountain Region, Forest Health Protection 2010), were found on both living, declining trees and dead snags. Saprotrophic decay fungi were prevalent with white-rot species predominating. These included *Hypholoma fasciculare* (Stand 7), the agaric “sulfur top” mushroom that is prolific on dead wood of both conifers and hardwoods

(Miller and Miller 2006). Other white rot fungi were *Hyphoderma setigerum* (Stands 8, 9, 13), *Trichaptum fuscoviolaceum* (Stand 11), and *Amylostereum chailletii* (Stand 18). These were all found on living, declining trees, with or without signs of insect colonization.

Many ophiostomoid sapstain (“blue stain”) fungi were detected, as expected for declining pines. These included *Ophiostoma pulvinisporum* (Stands 8, 9), *O. minus* (Stands 14, 22), *Leptographium lundbergii* (Stands 10), *Leptographium guttulatum* (Stand 10), *Graphium penicillioides* (Stand 30), and unknown species of *Ophiostoma* (Stands 8, 13, 18), *Ceratocystis* (Stands 3, 12), and *Graphium* (Stand 30). Most of these fungi were associated with dead trees (Stands 12, 22, 30) or signs of insect activity (Stands 10, 12). These fungal genera are frequently vectored by bark beetles and may be opportunistic pathogens (Gibbs 1993; Harrington 1993).

Table 1. Sampling site information.

Stand #	Date Sampled	State	County	Symptomatic tree #	DBH (")	Site Description
1	6/28/2011	Michigan	Kalkaska	1	12.8	Possible fruiting bodies
2	11/5/2011	Michigan	Leelanau	2	13.2	Pockets of decline clearcut; no dead trees
3	11/5/2011	Michigan	Benzie	1	8.5	Pockets of decline clearcut; no dead trees
4	11/5/2011	Michigan	Benzie	2	13.7	Pockets of decline clearcut; no dead trees
5	11/5/2011	Michigan	Benzie	1	10.2	Pocket started about 20 years ago; spread from 10-15 trees to several acres. Some dead trees very old and rotten.
6	11/5/2011	Michigan	Benzie	2	7.2	No information.
7	11/5/2011	Michigan	Leelanau	1	10.4	No information.
8	11/5/2011	Michigan	Leelanau	2	10.4	No information.
9	11/5/2011	Michigan	Leelanau	1	6.8	Pockets of decline. Dead trees clearcut to prevent spread.
10	11/5/2011	Michigan	Leelanau	2	7.2	Pockets of decline. Dead trees clearcut to prevent spread.
11	11/7/2011	Michigan	Mecosta	1	11.8	Large stand with 2 dead pockets.
12	11/7/2011	Michigan	Mecosta	2	10.8	Large stand with 2 dead pockets.
13	11/7/2011	Michigan	Mecosta	1	11.4	Large stand with 2 dead pockets.
14	5/23/2011	Minnesota	Anoka	2	11.3	Large stand with 2 dead pockets.
15	5/23/2011	Minnesota	Anoka	1	8.3	Stand of remnant pine on suburban lot adjacent to road. Several dead and dying trees.
16	5/23/2011	Minnesota	Anoka	2	8.8	Stand of remnant pine on suburban lot adjacent to road. Several dead and dying trees.
17	6/14/2011	Minnesota	Washington	1	11.6	Stand was located just off the east side of the parking lot of nature center building. Some wilting and dead red pine trees present.
18	6/16/2011	Minnesota	Sherburne	2	10	Stand was located just off the east side of the parking lot of nature center building. Some wilting and dead red pine trees present.
19	6/16/2011	Minnesota	Sherburne	1	10.1	Tress with dead needles and frass at base located on south side of a country road.
20	6/16/2011	Minnesota	Sherburne	2	14.1	Tress with dead needles and frass at base located on south side of a country road.
21	6/16/2011	Minnesota	Sherburne	1	10	<i>Ips</i> and red turpentine beetle present in stand located south of paved road.
22	6/16/2011	Minnesota	Sherburne	2	11.7	<i>Ips</i> and red turpentine beetle present in stand located south of paved road.
23	6/17/2011	Minnesota	Washington	1	6.9	Pockets of dead and dying trees located northeast of entrance building to park.
24	6/17/2011	Minnesota	Washington	2	6.2	Pockets of dead and dying trees located northeast of entrance building to park.
25	6/17/2011	Minnesota	Washington	1	14.9	Dead and dying trees located northeast of entrance building to park.
26	6/24/2011	Minnesota	Pine	2	10.7	Dead trees found stand located just east of a state highway.
27	11/4/2011	Wisconsin	Wood	1	10	Dead trees found stand located just east of a state highway.
28	11/4/2011	Wisconsin	Wood	2	11.3	Non-thinned plantation
29	11/4/2011	Wisconsin	Wood	1	5.5	Non-thinned plantation
30	11/4/2011	Wisconsin	Wood	2	6.1	Non-thinned plantation
31	11/15/2011	Wisconsin	Lincoln	1	10.2	Clump of 13 symptomatic trees with thin crowns and mortality. <i>Armillaria</i> rhizomorphs observed.
32	11/15/2011	Wisconsin	Lincoln	2	9.7	Clump of 13 symptomatic trees with thin crowns and mortality. <i>Armillaria</i> rhizomorphs observed.
33	11/17/2011	Wisconsin	La Crosse	1	12.3	Thinning crowns and dead trees approx. 60 years old.
34	11/17/2011	Wisconsin	La Crosse	2	11.1	Thinning crowns and dead trees approx. 60 years old.
35	11/17/2011	Wisconsin	Jackson	1	12.1	Horseshoe-shaped pocket; numerous trees with thinned crown.
36	11/17/2011	Wisconsin	Jackson	2	15.1	Horseshoe-shaped pocket; numerous trees with thinned crown.
37	11/17/2011	Wisconsin	Shawano	1	14	Established pocket of dead trees, on ground and standing.
38	11/17/2011	Wisconsin	Shawano	2	10	Established pocket of dead trees, on ground and standing.
39	12/1/2011	Wisconsin	Eau Claire	1	16	Dead trees and trees with thinning crowns.
40	12/1/2011	Wisconsin	Eau Claire	2	12	Dead trees and trees with thinning crowns.
41	12/1/2011	Wisconsin	Trempealeau	1	14	Stand of small saw timber.
42	12/1/2011	Wisconsin	Trempealeau	2	14	Stand of small saw timber.
43	12/2/2011	Wisconsin	Vilas	1	13	Large pocket of about 2 acres. Pre-salvaged ring of asymptomatic trees around symptomatic pines in 2009.
44	12/2/2011	Wisconsin	Vilas	2	14.2	Large pocket of about 2 acres. Pre-salvaged ring of asymptomatic trees around symptomatic pines in 2009.
45	12/2/2011	Wisconsin	Vilas	1	10.3	Approx. 20 extremely thin-crowned at edge of plantation. <i>Leptographium</i> , <i>Armillaria</i> and <i>Ips</i> sp. observed.
46	12/2/2011	Wisconsin	Vilas	2	10.5	Approx. 20 extremely thin-crowned at edge of plantation. <i>Leptographium</i> , <i>Armillaria</i> and <i>Ips</i> sp. observed.
47	12/2/2011	Wisconsin	Vilas	1	7.5	9 scattered pockets of mortality. Bark beetles, <i>Armillaria</i> , <i>Leptographium</i> observed. Pocket sampled had 14 symptomatic trees.
48	12/2/2011	Wisconsin	Vilas	2	10.7	9 scattered pockets of mortality. Bark beetles, <i>Armillaria</i> , <i>Leptographium</i> observed. Pocket sampled had 14 symptomatic trees.
49	12/6/2011	Wisconsin	Monroe	1	9.7	Red/white pine plantation approx. 50 years old. White pine healthy and regenerating.
50	12/6/2011	Wisconsin	Monroe	2	7.3	Red/white pine plantation approx. 50 years old. White pine healthy and regenerating.
51	12/6/2011	Wisconsin	Monroe	1	5.6	Red/white pine plantation approx. 50 years old. Dead, declining trees with tufted foliage. WP scattered in overstory.
52	12/6/2011	Wisconsin	Monroe	2	6.1	Red/white pine plantation approx. 50 years old. Dead, declining trees with tufted foliage. WP scattered in overstory.
53	12/8/2011	Wisconsin	Washington	1	9.5	Red/white pine plantation approx. 40 years old. Scattered dead and declining trees, rapid mortality. Southern Pine Engraver and <i>Ips grandicollis</i> suspected.

Table 2. Potential pathogens, decay and sapstain fungi recovered by location^A.

Stand #	County	State	Tree # or Control	Symptoms / Signs Observed	Drill 1	Drill 2	Drill 3	Drill 4
3	Benzle	MI	2	Dead with bark still attached	<i>Ceratocystis</i> sp.			
7	Mecosta	MI	1	Recently dead tree near pocket edge			<i>Hypholoma fasciculare</i>	<i>Hypholoma fasciculare</i>
8	Anoka	MN	1	Thinning crown	<i>Ophiostoma pulvinisporum</i>			<i>Valsa</i> sp.
			2	Thinning crown	<i>Ophiostoma</i> sp. B <i>Hyphoderma setigerum</i>			<i>Valsa</i> sp. <i>Phoma</i> sp.
9	Washington	MN	1	Wilted needles attached	<i>Hyphoderma setigerum</i>		<i>Hyphoderma setigerum</i>	
			2	Wilted needles attached	<i>Ophiostoma pulvinisporum</i>		<i>Ophiostoma pulvinisporum</i>	<i>Hyphoderma setigerum</i>
10	Sherburne	MN	1	Dead needles with frass at base	<i>Leptographium lundbergii</i>		<i>Leptographium guttulatum</i>	
11	Sherburne	MN	1	<i>Cryptoporus</i> and red turpentine beetle	<i>Leptographium lundbergii</i>		<i>Leptographium guttulatum</i>	
			1	Woodpecker damage, <i>Ips</i> sp.	<i>Botryobasidium subcoronatum</i>			<i>Trichaptum fuscoviolaceum</i>
			1	"Healthy" control tree	<i>Daedalopsis confragosa</i>			
			1	Dead, <i>Ips</i> sp.	<i>Nectria nigrescens</i>	<i>Ceratocystis</i> sp. B		
			2	<i>Ips</i> sp. holes, wilting needles	<i>Nectria nigrescens</i>			
13	Washington	MN	1	<i>Ips</i> sp. holes, <i>Armillaria</i> , red turpentine beetle	<i>Hyphoderma setigerum</i>	<i>Hyphoderma setigerum</i>		
			2	<i>Ips</i> sp. holes, <i>Armillaria</i> , red turpentine beetle	<i>Hyphoderma setigerum</i>	<i>Hyphoderma setigerum</i>	<i>Ophiostoma</i> sp.	<i>Hyphoderma setigerum</i>
14	Pine	MN	1	Root collar weevil, resinous wood		<i>Armillaria solidipes</i>	<i>Leptographium guttulatum</i>	<i>Ophiostoma minus</i>
			2	Discolored wood				
15	Wadena	MN	1	Dead	<i>Nectria mariamaeae</i>			
			2	Sawdust at base indicating borers			<i>Nectria mariamaeae</i>	
17	Wood	WI	1	Thin crown, tufted needle tips, chlorotic needles		<i>Coniophora</i> sp.		
			2	Thin crown, tufted needle tips, chlorotic needles	<i>Coniophora puteana</i>			
18	Lincoln	WI	1	Dead top, 40% necrotic needles & thin crown, <i>Ips</i>	<i>Amylostereum chianitii</i>	<i>Ophiostoma</i> sp.	<i>Amylostereum chailletii</i>	<i>Parasola</i> sp.
			2	Thin crown		<i>Ophiostoma</i> sp. A		<i>Ophiostoma</i> sp.
20	LaCrosse	WI	2	Very thin crown, tufted needles	<i>Coniophora puteana</i>	<i>Coniophora puteana</i>	<i>Ophiostoma</i> sp.	<i>Coniophora puteana</i>
22	Shawano	WI	C	"Healthy" control tree		<i>Scytinostroma</i> sp.	<i>Coniophora puteana</i>	
			1	Dead		<i>Coniophora arida</i>		
			2	Dead	<i>Scytinostroma</i> sp.		<i>Ophiostoma minus</i>	
						<i>Scytinostroma</i> sp.	<i>Scytinostroma</i> sp.	
24	Trempealeau	WI	1	Thin, tufted foliage	<i>Coniophora arida</i>	<i>Coniophora arida</i>		<i>Scytinostroma</i> sp. A
25	Vilas	WI	1	Stump with bark attached				<i>Scytinostroma</i> sp. B
								<i>Scytinostroma</i> sp. C
26	Vilas	WI	1	Dead	<i>Armillaria solidipes</i>	<i>Armillaria solidipes</i>		
27	Vilas	WI	2	Dead	<i>Nectria mariamaeae</i>	<i>Nectria mariamaeae</i>		
28	Monroe	WI	C	"Healthy" control tree	<i>Sistotrema semanderi</i>			
			2	Thin, tufted foliage	<i>Polyporales</i>	<i>Mycena sanguinolenta</i>		<i>Mycena sanguinolenta</i>
29	Monroe	WI	2	Thinning crown, tufting foliage	<i>Mycena sanguinolenta</i>			
30	Washington	WI	1	Dead			<i>Graphium penicillifoloides</i>	<i>Graphium</i> sp.
			2	Bark beetle	<i>Nectria flavoviridis</i>	<i>Graphium</i> sp.	<i>Botryobasidium botryosum</i>	<i>Graphium</i> sp.
					<i>Hebeloma mesophaeum</i>			<i>Graphium</i> sp.

^A Stands with no identified fungi are excluded from this table.

Three species of *Nectria*, the genus responsible for Nectria canker and Nectria dieback, were also common on the dead and declining *P. resinosa* (Stands 12, 15, 27, 30). *Nectria mariannaea*, *N. nigrescens*, and *N. flavoviridis* are saprotrophs or endophytes not associated with disease that are commonly found on bark and woody substrates of numerous hosts, sometimes only in their imperfect state (Hirooka et al. 2011; Samuels and Seifert 1991). Identification to exact species based on ITS data is not possible, however. *Nectria nigrescens*, which has been associated exclusively with hardwoods, is part of the *Nectria cinnabarina* species complex (NCSC). Other members of the NCSC have been collected from conifers including the Pinaceae (Hirooka et al. 2011) and may be difficult to differentiate using only ITS primers. *Nectria mariannaea* has only been reported from French Guiana and Venezuela, but its anamorph, *Stilbella aciculosa*, is distributed widely throughout Europe, North America, Asia and the tropics (Samuels and Seifert 1991). *Nectria flavoviridis* is often a mycoparasite (Ellis and Ellis 1988) so it could be colonizing other ascomycetous saprotrophs.

Numerous ascomycetes, including endophytic fungi and yeasts, were recovered in great quantities (data not shown). Many of these were uncommon and site specific. Thirty-two unique sequences from basidiomycetes and 129 from ascomycetes had no species, genus, or family identification in GenBank, thus illustrating the complex and largely unstudied community of fungi found in declining pines.

Fungal DNA was not detected in any of the air samples. Control “healthy” trees contained large numbers of nonpathogenic fungi, including endophytes, yeasts, and discomycetes, as well as many “unknown” ascomycetes that have not been identified in GenBank. Two control trees contained DNA associated with decay fungi. In Stand 12, the white rotter *Daedalopsis confragosa* was detected in one drill sample. This was unexpected because the fungus is found only rarely on conifers (Gilbertson & Ryvarden 1986) and the tree was asymptomatic. In Stand 22, one drill sample from the asymptomatic control tree contained DNA from *Scytinostroma* sp., a root rotting white-rot fungus that was prevalent in the sampled trees from that plot.

Field personnel were surveyed to determine their opinions regarding the drilling technique. Many saw its value for research but felt that it was somewhat time-consuming compared to normal field sampling techniques. This observation may have been due to individuals’ unfamiliarity with the technique and the fact that each individual only collected a small number of samples. Interestingly, many recognized the value of being able to identify a wide range of fungal pathogens in sites where fruiting bodies could not be located.

No single fungal pathogen was found to dominate samples in sites where obvious fruiting bodies were not present. *Armillaria solidipes* (= *A. ostoyae*) could be a contributor to tree decline in certain stands but is usually associated with trees that are already stressed by other agents in the Northeast and Midwest (Sinclair and Lyon 2005). Another possible mortality agent is *Scytinostroma galactinum*. This fungus is frequently associated with ash, apples, and other hardwoods but has also been known to cause root rot, butt rot, and heart rot of conifer species, including *P. resinosa* and *P. banksiana*, in Wisconsin and Ontario (Krebill 1963; Basham and Morawski 1964). Reports of its pathogenicity are summarized in Lentz and Burdsall (1973). The prevalence of this fungus and its role in the unexpected dying of red pine in the Lake States needs to be examined further.

Two species of *Leptographium*, *L. lundbergii* and *L. guttulatum*, were detected in the survey, but these are not the species typically associated with RPPM (Klepzig et al. 1991). They are sapstain fungi, however, and are likely vectored to stressed trees by beetles. Many of the sampled trees exhibited signs of insect activity – frass, emergence holes, and beetle galleries. *Ophiostoma ips* is a major contributor to RPPM and is often responsible for ultimate tree death along with its insect associate (Klepzig et al. 1991). Although *O. ips* was not identified, other species of *Ophiostoma* were found, including *O. minor* which has been associated with RPPM (Klepzig et al. 1991). Other isolates of *Ophiostoma*, *Graphium*, and *Ceratocystis* could not be identified to species without the use of additional primers and may be species associated with RPPM. The role of abiotic factors, including drought and poor soil types, and the attraction of insects to weakened trees are also possible contributors to the observed declines.

As we were assaying with both *H. irregulare*-specific and nonspecific fungal primers, the laboratory analysis was quite time-consuming but resulted in much additional information on fungi associated with dying *Pinus resinosa* in affected stands. The procedures presented here can be used to address questions of fungal diversity and ecology as well as forest pathology. The technique can also be easily adapted to screening with species-specific primers. Sampling fungal DNA directly from wood removes the extensive labor and biases associated with culturing. The sensitivity of the assay would allow drill samplings from a tree or even an entire stand to be pooled, as recommended by Guglielmo et al. (2010), and thus greatly speed identification and decrease laboratory costs. These procedures have many potential applications in forest pathology for both applied survey work and basic research studies.

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SUDDEN OAK DEATH MANAGEMENT AND CONTAINMENT STRATEGIES IN THE NORTH COAST OF CALIFORNIA

Yana Valachovic¹ and Maia Beh¹

In California, sudden oak death (SOD) treatment efforts have been localized, often targeting specific trees or properties. The widespread nature of *Phytophthora ramorum* (causal agent of SOD) establishment and spread in California has mostly precluded use of broader eradication and containment strategies, which are more applicable in isolated infestations like those in Oregon. However, the 2010 detection of a new infestation in Redwood Valley, CA – more than 50 miles from the nearest infestation, and the northernmost occurrence in the state – presented an opportunity for the first large-scale containment effort in California. The infestation was isolated to a relatively small geographic area and was of high priority, effectively located at the gateway to Redwood National Park, Yurok and Hoopa tribal lands, Bureau of Land Management and USDA Forest Service lands, and the dense tanoak forests of the Klamath watershed.

Our disease containment efforts in Redwood Valley have involved treatment of 370 acres during this first phase of the containment efforts. Treatment work involved the removal of all *P. ramorum*-infected trees as well as those species that support ample sporulation of the pathogen (California bay laurel and tanoak) within 100 meters of an infected tree. Cut plant material was securely trucked offsite and donated to a nearby wood-fired power generation plant, piled and burned, or lopped and scattered onsite. We are currently in a post-treatment monitoring phase in Redwood Valley, in which we survey the forests just outside of our treatment area to determine if *P. ramorum* has “leaked” outside of the treatment perimeter. This important phase of the project will help us gauge the efficacy of our containment efforts and map any recent expansions of the pathogen into surrounding forests. The results of our surveys will also set the stage for our next course of

action: planning for additional treatments if the pathogen has escaped containment, or continued intermittent surveys if the treatment perimeters hold.

The strategy in Redwood Valley has been shaped by our experiences from several prior silvicultural experiments to manage SOD. For example, in 2006, three forested sites in Humboldt County infested with *P. ramorum* were subjected to different combinations of treatments designed to reduce inoculum and control spread. Treatments included the following: (1) cutting of California bay laurel and tanoak trees; (2) cutting of all bay and tanoak followed by broadcast burning to assist in reducing host saplings and seedlings; (3) the above treatments combined with removal of Pacific madrone saplings to assess the effect of removing this additional *P. ramorum* host; (4) removal of bay laurel alone by chainsaw; (5) girdling of bay laurel alone; and (6) treatment of bay laurel and tanoak by herbicide alone to kill the standing tree and control sprouting. Treatments have been monitored for 7 years, and results to date suggest that the treatments that involved the cutting of bay laurel and tanoak substantially reduced *P. ramorum* inoculum levels. However, in treatment areas where scattered bay trees were inadvertently missed because of the limited window for harvest operations owing to *marbled murrelet* (*Brachyramphus marmoratus*) nesting season restrictions, we have observed that a relatively minor component of residual bay laurel trees may have become infected following treatment and subsequently spread *P. ramorum* to regenerating bay and tanoak sprouts and seedlings. The data suggest that pathogen reestablishment was driven by both incomplete treatment application and spread from adjacent, but untreated stands.

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A NEW DIMENSION TO PITCH CANKER EPIDEMIOLOGY: BIOLOGY OF *FUSARIUM CIRCINATUM* AS A GRASS COLONIST IN NATIVE AND MANAGED PINE SYSTEMS

Cassandra L. Swett¹, Margaret Huang¹, Amir Begovic¹, and Thomas R. Gordon¹

ABSTRACT

The host range of the pitch canker pathogen *Fusarium circinatum*, long thought to be limited to conifers, had recently been extended to include grasses. The objectives of this study were to assess the potential impact of infected grasses on the epidemiology of pitch canker in native *Pinus radiata* and *P. muricata* populations in California, and in managed stands of *P. radiata* in South Africa. To this end, we identified a wide range of species harboring the fungus in field surveys in both California and South Africa and in greenhouse trials. We also characterized strain diversity in grasses, confirmed the ability for grass-associated isolates to infect pines, and found that the fungus is capable of asexual reproduction on senescing shoot tissue. Together these results indicate that a wide range of grass species can provide a cryptic source of inoculum capable of facilitating development and spread of pitch canker in both native and managed pine systems worldwide.

INTRODUCTION

Pitch canker is a disease of conifers, mostly pines. The disease has major impacts on native pines in coastal populations in California, and in pine production systems around the world, especially in South Africa (Gordon 2012; Wingfield et al. 2008). Since its initial description in 1946, the known host range for *F. circinatum* has been limited to the Pinaceae (*Pinus* spp. and *Pseudotsuga menziesii*) (Gordon 2006; Dwinell 1985; Hepting and Roth 1945). However, this pathogen is closely related to the grass colonizer *Fusarium subglutinans*, based on interfertility between species and gene phylogenies (Gordon 2012). If these two species diverged from a grass colonizing ancestor, modern populations of *F. circinatum* may partially retain ancestral grass colonizing abilities. In support of this hypothesis, recent studies have shown that *F. circinatum* can naturally infect several grass species in native California forests (Swett and Gordon 2012). In preliminary studies with *Zea mays*, *F. circinatum* is capable of the same modes of horizontal and vertical transmission as known *Fusarium* grass colonists, and does not appear to have any negative host effects (Swett and Gordon 2009).

The objectives of this study were to examine the significance of grass infecting abilities on pitch canker epidemiology in native *Pinus radiata* and *P. muricata* populations in California, and extend these findings to managed *P. radiata* plantations in South Africa. In the California studies, we evaluated the potential host range on grasses in California in surveys and greenhouse trials, the diversity of strains recovered from grasses in two coastal populations, the virulence of these strains on *P. radiata*, and the ability for the species to asexually reproduce on grass. To extend these findings to managed systems, we conducted a survey of grass species surrounding/within plantation and nursery-grown pines along the Western Cape in South Africa.

MATERIALS AND METHODS

Studies of grass infection in native forests in California were conducted in two locations along the California coast line, in native stands of *Pinus radiata* (Monterey pine) on the Monterey Peninsula and *P. muricata* (bishop pine) at Pt. Reyes National Seashore, during July and August of 2011. Morphologically diverse grasses were collected from beneath infested trees at three to five sites at each location. Leaves and stems were rinsed in 0.1 percent Tween 20, immersed in 70 percent ethanol for 10 seconds followed by 1 minute in 1 percent NaOCl, and placed on a *Fusarium* selective medium (FSM) (Aegerter and Gordon 2006). Colonies growing from cultured plant material which resembled *F. circinatum* were single spore sub-cultured onto

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carnation leaf agar and 0.1 percent KCL agar and tentatively identified based on microscopic examination of colony morphology, using the description of Leslie et al. (2006).

A known *F. circinatum* from pines (Fsp 17) and one isolate each from *Festuca arundinaceae* and *Holcus lanatus* were tested for their ability to infect *F. arundinaceae* variety Fawn, and four grass species native to California or the west coast (*Bromus carinatus*, *Elymus glaucus*, *Festuca idahoensis* and *Festuca rubra*). For each isolate, twenty 14 day-old seedlings (ten pots with two plants per pot) were sprayed to run-off with an aqueous suspension of 10^6 spores per ml. All inoculations were repeated. Two weeks after inoculation, leaves and stems were rinsed briefly in 0.1 percent Tween 20, immersed for 10 seconds in 70 percent ethanol, followed by 30 seconds in 1 percent NaOCl, and cultured on FSM.

Three isolates from each source (nine total), and the known *F. circinatum* isolate from pines (Fsp 17) were tested for virulence on one year old greenhouse-grown Monterey pine clones. Three trees were inoculated with each isolate by inserting 250 spores in a shallow 1.6 mm stem wound and lesion length measured two weeks after inoculation.

To characterize the strains of *F. circinatum* recovered from grasses, somatic compatibility was analyzed for all isolates from *F. arundinacea* and *H. lanatus* using tester strains for all vegetative compatibility groups known to occur in California, following the protocol outlined in Gordon et al. (1996).

Analysis of sporulation abilities was conducted by surface disinfesting healthy-looking leaves (as described above) from *F. arundinaceae* inoculated six weeks previously (as described above), and incubating leaves in sterile glass incubation chambers, on a moisten filter paper. Sporulation was examined for two isolates: a pine isolate transformed to express a GFP tag (prepared based on the protocol of Covert et al. 2001) and an isolate from grasses. Development of sporulating colonies was visualized under an epifluorescent and compound microscope for the two isolates respectively. At the time sporulation occurred, leaf condition was rated as either healthy (green), early senescence (yellowing) and advanced senescence (browning), and

all emerging colonies were subcultured first to FSM and then to 0.1 percent KCL for identification (as described above).

Surveys of grass associations in South Africa were conducted in a plantation along the Western Cape and a nursery in the Mpumalanga province. Twenty eight grass species total were collected from within select areas with high pitch canker incidence, and isolation and identification were conducted as described above. Isolates with similar morphology to *F. circinatum* were further identified based on sequence analysis. Fungal DNA was extracted from pure culture, using the PrepMan Ultra DNA extraction kit (Applied Biosystems) and the TEF1- α region was amplified by PCR using EF1 and 2 forward and reverse primers under the following parameters: 95°C for 5 minutes, followed by 35 cycles of 92°C for 1 minute, 53°C for 1 minute, 72°C for 1 minute and a final extension at 72°C for 10 minutes. The amplified gene products were then sequenced with both the forward and reverse primers, and these sequences were used for BLAST search analysis in the Fusarium-ID database (<http://isolate.fusariumdb.org/index.php>). Taxon identity was assigned based on 98 percent or greater homology with database sequences.

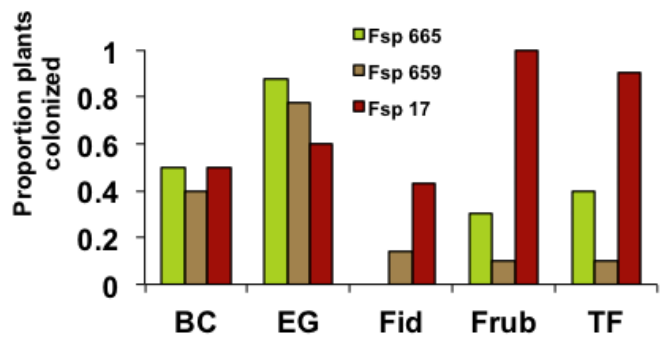


Figure 1. Colonization of *F. circinatum* in inoculation trials with native and non-native grass species. Native California species: BC=*Bromus carinatus*, Eg=*Elymus glaucus*, Fid=*Festuca idahoensis*, Frub=*Festuca rubra*; Non-Native species common in California: TF=*F. arundinaceae*.

RESULTS

Putative *Fusarium circinatum* isolates were recovered from six grass species on the Monterey Peninsula

(*Holcus lanatus*, *Festuca arundinaceae*, *Briza maxima*, *Calamagrostis koeleriodes*, *Agrostis gigantia*, and *Agrostis stolonifera*) and one species at Pt. Reyes National Seashore (*H. lanatus*) (Table 1). In greenhouse infection trials, *F. circinatum* was able to colonize all grass species tested; isolates were recovered from at least some of the inoculated plants (range = 20-100 percent), from living stems and leaves, as well as from senescing tissue (Figure 1). In pine inoculation trials, all grass isolates had induced resinous branch cankers, chlorosis of surrounding needles, and lesions comparable in length (17 – 24 mm) and appearance to those caused by Fsp 17, two weeks after inoculation.

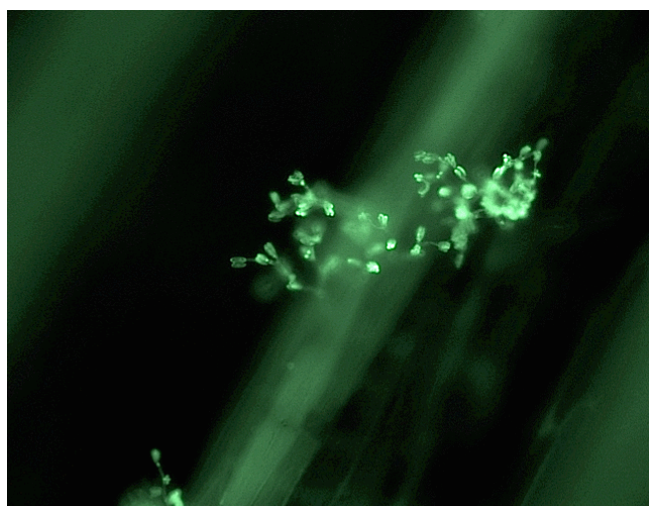


Figure 2. Production of conidia by GFP tagged *F. circinatum* isolate (visualized at 20x on a Zeiss epifluorescent microscope).

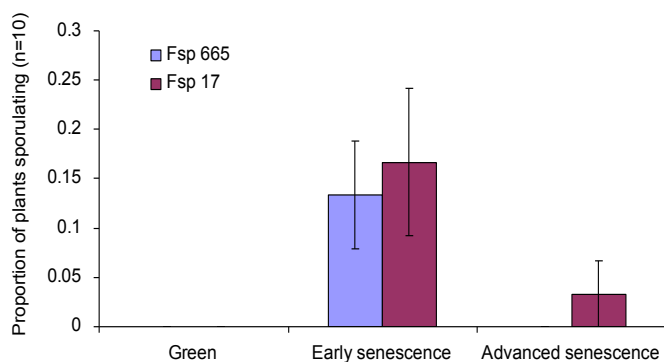


Figure 3. Proportion of inoculated, surface disinfested plants with sporulating colonies, at each stage of senescence within incubation chambers.

Strains from grasses in California were predominately homologous with strains from pines with the potential exception of one strain that did not pair with any of the known somatic compatibility groups in California. The recovered isolates were pathogenic on pine, with equal virulence to an isolate from pines. The ability of the fungus to asexually reproduce from internally infected grass tissue was confirmed based on observations of sporulating colonies using epifluorescent microscopy (Figure 2); sporulation occurred on 70 percent of grass leaves exposed to the fungus, and only began after leaves started to senesce, suggesting that, in addition to its endophytic association in grasses, *F. circinatum* is also an opportunistic saprophyte (Figure 3).

In surveys in South Africa, *F. circinatum* was recovered from four of the twenty-eight species surveyed (*Briza maxima*, *Eragrostis biflora*, *Pentameris pallid*, and one unknown species), only from the plantation, verifying that this grass association has relevance both ecologically and commercially.

Table 1. Grass species associated with *F. circinatum* in native Pine forests along the California coast.

Grass species	Location/s
<i>Festuca arundinacea</i>	Monterey
<i>Holcus lanatus</i>	Point Reyes & Monterey
<i>Briza maxima</i>	Monterey
<i>Calamagrostis koeleriodes</i>	Monterey
<i>Agrostis gigantia</i>	Monterey
<i>Agrostis stolonifera</i>	Monterey

CONCLUSIONS

These results suggest that grass species can be symptomless hosts for *F. circinatum*, constituting the first documentation of any non-Pinaceae host for this pathogen. As cryptic reservoir hosts, inoculum from infected grasses may influence disease development and spread into new areas. Together these results indicate that a wide range of grass species can provide cryptic reservoirs of inoculum capable of facilitating development and spread of pitch canker in both native and managed pine systems worldwide. Studies are underway to further characterize the extent of the host range of *F. circinatum* outside of the pine genus and examine the epidemiological implications of grasses as alternate hosts.

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POSTER PAPERS AND ABSTRACTS





THE EFFECT OF MOUNTAIN PINE BEETLE AND DWARF MISTLETOE ON CANOPY STRUCTURE AND POTENTIAL CROWN FIRE BEHAVIOR IN CENTRAL OREGON LODGEPOLE PINE FORESTS

Michelle C. Agne¹, Travis J. Woolley¹, and David C. Shaw¹

ABSTRACT

For a previous project, 208 plots were installed in Central Oregon lodgepole pine (*Pinus contorta*) stands which experienced a mountain pine beetle (*Dendroctonus ponderosae*) epidemic between 2 and 31 years previously. In these stands there was a 72 percent incidence of dwarf mistletoe. These results suggested that dwarf mistletoe is an important component of lodgepole pine forests following a mountain pine beetle epidemic and should be further investigated.

The purpose of this study is to investigate the impacts of dwarf mistletoe on canopy structure in lodgepole pine forests in Central Oregon using two objectives: (1) Understanding differences in canopy structure between post-mountain pine beetle lodgepole pine stands influenced by dwarf mistletoe and those that are not, and (2) predicting fire behavior in these stands using information about canopy structure. This study expands on previous findings regarding canopy structure in dwarf mistletoe infested stands through greater replication and plot randomization. Additionally, this study accounts for the impact that mountain pine beetle has on the structure of lodgepole pine forests. By considering stands from only one stage in the original chronosequence, the variability in stand structure that can be attributed to the mountain pine beetle is minimal.

The study area included thirteen climax lodgepole pine stands from 21 to 28 years post mountain pine beetle epidemic located in Central Oregon. Canopy structure characteristics were quantified for mature trees within three randomly located plot replicates in each stand. Each tree was also assessed for dwarf mistletoe using the Hawksworth 6-class rating system as well as a percent broom volume rating system. Preliminary results suggest that increased stand level dwarf mistletoe infestation may lower overall canopy base height within lodgepole pine stands, however further analysis is required to validate this claim fully.

Following analysis of the effect of dwarf mistletoe on canopy structure, the fire behavior modeling program BehavePlus will be used to determine whether the change in structure caused by dwarf mistletoe infestation has a significant impact on fire behavior. Two structural components hypothesized to be impacted significantly by dwarf mistletoe (canopy base height and canopy bulk density) are two important variables when determining the transition of a surface fire to a crown fire. Although there is a common belief that increased dwarf mistletoe infestation leads to higher likelihood of crown fire, this has never been shown empirically.

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ASSESSING LIMBER PINE STAND CONDITIONS AFTER BLISTER RUST AND MOUNTAIN PINE BEETLE OUTBREAKS IN THE CENTRAL AND SOUTHERN ROCKY MOUNTAINS

Christy Cleaver¹, William Jacobi¹, Kelly Burns², and Bob Means³

ABSTRACT

Mountain pine beetle and white pine blister rust are causing extensive crown dieback and mortality in limber pine (*Pinus flexilis* James) in the Central and Southern Rocky Mountains. Ecologically valuable limber pines often grow in fragile ecosystems where few other trees can grow. The combined effects of mountain pine beetle, white pine blister rust, and climate change could greatly impact the biodiversity of these ecosystems. Information on stand conditions is needed to facilitate management and restoration efforts. The objectives of this study are to: (1) determine the density and health of mature limber pine trees and regeneration in areas impacted by white pine blister rust and mountain pine beetle on BLM and National Forest lands in northern Colorado, Wyoming, and Montana, and (2) determine factors that impact regeneration, including site and stand characteristics, white pine blister rust, and mountain pine beetle.

In 2011, we assessed limber pine stands in eight mountain ranges in Northern Colorado and Wyoming. One hundred seventy-five previously monitored stands were revisited and plots were established. The incidence of white pine blister rust was greatest (32 to 34 percent) in the Pole Mountain (WY), Green Mountains (WY), and Shirley Mountains (WY) plots. The Northern Front Range (CO) and the north and south sections of the Medicine Bow Range (WY) each had less than 5 percent of limber pine stems infected with white pine blister rust. No infected trees were detected in the Sierra Madre Range (WY). Incidence of infection was greatest on limber pine regeneration in the Green Mountains, Pole Mountain, and Shirley Mountains where 3 to 4 percent of regeneration less than 70 cm tall were infected and 8 to 16 percent of regeneration equal to or greater than 70 cm tall were infected. No infections were detected on regeneration in the north and south sections of the Medicine Bow Range or the Sierra Madre. The Medicine Bow Range and Sierra Madre each had the greatest percent of all pine species with evidence of bark beetle infestation at 29 and 26 percent, respectively. In 2012, we assessed limber pine stands in seventeen other mountain ranges in Northern Colorado, Wyoming, and Montana and established three hundred thirty-three plots. Future data analysis and modeling will look for relationships to explain successful limber pine regeneration, the impact of rust and bark beetle mortality on overstory trees and regeneration, and provide management information to maintain limber pine in the forest ecosystem.

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ASPEN MORTALITY IN THE COLORADO AND SOUTHERN WYOMING ROCKY MOUNTAINS: EXTENT, SEVERITY, AND CAUSAL FACTORS

Megan Dudley¹, William Jacobi¹, and Kelly Sullivan Burns²

ABSTRACT

Quaking aspen (*Populus tremuloides* Michx.) dieback has been documented throughout western North America over the past decade, resulting in stands that have either elevated proportions of overstory mortality or thin crowns, or both. Stands experiencing dieback may or may not produce regeneration cohorts. In this study, we surveyed aspen in the north-western corner of Colorado on the White River and Routt national forests, along the front range of Colorado on the Pike-San Isabel national forests, and in the south-central region of Wyoming on the Medicine Bow national forest during 2009 - 2010.

We established 573 random roadside survey plots in stands that contained at least 50 percent aspen stems. From these random plots, we found average standing aspen tree mortality ranged from 3.3 to 23.7 percent on the four national forests. Mortality averaged 11 percent among plots east of the continental divide and 4 percent on the west side. The aspen on the four national forests surveyed were healthy overall, with low average percent crown dieback among adult aspen (~10 – 15 percent), in spite of nearly ubiquitous presence of disease (~97 – 99 percent) and high incidence of insect damage (~50 – 75 percent) among survey plots.

We also established 98 stand assessment plots with half of the plots in damaged aspen stands, as defined by U.S.D.A. Forest Service aerial detection surveys, and half of the plots in healthy aspen stands. Damaged stands were defined as those stands with (1) thinning crowns among at least 25 percent of adult aspen, (2) stands with moderate (<50 percent of stems) levels of overstory mortality, or (3) stands with high (>50 percent of stems) levels of overstory mortality. Healthy aspen stands were defined as having (1) a maximum mortality rate of 5 – 7 percent among all aspen, and/or (2) more than 75 percent of adult aspen with full crowns. Adult aspen in damaged stands tended to be less vigorous, based on percent dead crown. Adults in the damaged stands averaged 38 percent dead crowns, and those in healthy stands averaged 14 percent dead crowns.

There was no difference in the proportion live or total numbers of saplings per hectare between healthy and damaged stands. The prevalence of damaging organisms, such as *Cytospora* canker (20 percent in damaged, 13 percent in healthy), wood-boring insects (27 percent in damaged, 10 percent in healthy), and aspen bark beetles (*Proccryphalus mucronatus* and *Trypophloeus populi*) (16 percent in damaged, 7 percent in healthy) was considerably greater among damaged stands. Site conditions also influenced the prevalence of some of these damage agents: bark beetles were most common among stands at low elevations (18 percent, compared to 11 percent and 6 percent at moderate and high elevations, respectively); *Cytospora* canker was most common among stands on south- or west-facing aspects (20 percent and 19 percent, respectively); both aspen bark beetles and *Cytospora* canker were also most common among stands in the southernmost section of the survey area, the Pike-San Isabel national forest (41 percent and 36 percent). There was no difference in the severity of canker or decay fungal infection between healthy and damaged stands.

Cytospora canker infestations were more severe on the Medicine Bow NF compared to the other three national forests, and *Marssonina* foliar blight infection appeared to be most severe on slope summits, concave sites, and sites with either no to low percent slope or moderately steep slopes.

Based on the general state of aspen health within the study area, it seems likely that localized environmental conditions (i.e. drought, late spring frost), coupled with disease and insect infestations, resulted in greater mortality when compared to healthy stands.

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The severity of such conditions appears to be regional in scale, and it remains to be established whether long-term or acute drought is the major factor influencing the observed conditions. Since no differences were detected in regeneration density between damaged or non-damaged stands, it is possible that there will be no long-lasting effects on aspen longevity on these sites with the relatively low incidence of overstory mortality throughout all four national forests.

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THE CULTURE COLLECTION AND HERBARIUM OF THE CENTER FOR FOREST MYCOLOGY RESEARCH: A NATIONAL RESOURCE

J.A. Glaeser¹, K.K. Nakasone¹, D.J. Lodge¹, B. Ortiz-Santana¹, and D.L. Lindner¹

EXECUTIVE SUMMARY

- The Center for Forest Mycology Research (CFMR), U.S. Forest Service, Northern Research Station, Madison, WI, is home to the world's largest collection of wood-inhabiting fungi.
- These collections constitute a library of the fungal kingdom that is used by researchers throughout the world.
- The CFMR collections have many practical uses that have improved the lives of Americans directly and indirectly over the past century in many ways.
- The CFMR and its collections contribute to tools to: identify and manage devastating fungal diseases of trees and wildlife, understand and maintain endangered wildlife populations that rely on fungi, provide fungal cultures to identify important pharmaceuticals and biotechnological processes, and develop sustainable forest management guidelines for bioenergy harvests from forests.

The Center for Forest Mycology Research (CFMR), U.S. Forest Service, Northern Research Station, Madison, WI, is home to the largest collection of wood-inhabiting fungi in the world. The culture collection includes approximately 20,000 living cultures of 1,800 species of fungi. The associated herbarium contains approximately 50,000 dried specimens of 4,200 species with many specimens dating back to the early 1900s. These collections constitute a library of the fungal kingdom that is used by researchers worldwide to classify, identify, and develop genetic profiles of wood-inhabiting fungi. Fungal identification is a difficult process, and these collections of living and dried organisms serve as reference standards for describing species, determining how much variability occurs within species, the extent of interbreeding among populations and species, and providing information on species' ecology and distribution. Mycologists have historically used macroscopic and microscopic features for fungal identification, augmented by culture morphology for wood-inhabiting fungi. Recently, molecular techniques involving DNA sequencing have become important tools aiding fungal identification (Glaeser and Linder 2011), but collection-based reference standards and DNA sequences based on those reference collections are still necessary. Whenever a

new fungus is encountered, it is formally described based on a "type specimen." A type is a reference specimen used as a standard to identify a species, often decades after it was originally described. The CFMR collection contains approximately 230 type specimens of important forest pathogens and decay fungi.

The CFMR collections have many practical uses that have directly and indirectly improved the lives of Americans over the past century. The identification of fungi associated with hardwood and conifer diseases was essential for the early forest pathologists of the 20th century (Davidson and Campbell 1943; Davidson et al. 1942). Working with forest managers, they developed management plans that reduced losses from disease and decay in forests being grown exclusively for timber production. During WWII, defects in lumber caused by decay fungi were identified, evaluated and cataloged so that defective wood was not used in the manufacture of wooden ships and airplanes for the war effort (Davidson et al. 1947). Early work on decay fungi identified species that destroyed buildings, telephone poles, railroad ties, mine-timbers, and other forest products, leading to efforts to develop wood preservatives that prevent fungal decay (Duncan and Lombard 1965; Zabel et al. 1985). Current wood preservation evaluation tests continue to use CFMR cultures as standards for testing new, environmentally friendly preservative treatments (AWPA 2012). CFMR cultures have also been used to develop bioremediation

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protocols to break down toxic waste (Lamar et al. 1993; Lamar et al. 1994) and for biopulping (Kirk et al. 1993), a process for paper pulp production that could potentially reduce the amount of toxic chlorinated hydrocarbons in the effluent.

Identification and detection of current disease and decay fungi and potential threats from invasive species are essential to preserve and manage American forests even as the goal of management has expanded from timber production and watershed protection. The U.S. National Forests now supply many benefits to a variety of customers, including recreation, wildlife management, carbon sequestration, and purification of air and water. The mission and users of the CFMR collections have changed as well.

Fungi are now known to be an important food source for small mammals, which in turn are food for larger predators. Understanding the fungal composition in a forest stand allows wildlife biologists to estimate and better manage wildlife populations. One example of this is the preferential consumption of underground fungi, commonly known as “truffles,” by flying squirrels in the Pacific Northwest. Flying squirrels foraging for truffles on the ground are vulnerable to predation by spotted owls and are the owls’ primary food source. Understanding and maintaining truffle populations has therefore been identified as a critical aspect of to protect and manage spotted owl habitat (Waters et al. 2000). Another example of the importance of fungi to wildlife populations is the relationship between heart-rot decay fungi and cavity-nesting birds, such as the endangered Red Cockaded Woodpecker. CFMR scientists are currently working with ornithologists at Virginia Tech to identify and enumerate the succession of decay fungi necessary for nest cavity formation (Jusino 2011), expanding upon earlier work (Conner et al. 1976). Nest cavity formation greatly affects the reproductive success and population distribution of the bird – parameters that must be factored into management plans of southern forests.

Certain fungi cause wildlife diseases that can significantly impact important animal populations. One of the most devastating examples of this in recent years is White Nose Syndrome of bats, caused by the fungus *Geomyces destructans*. Thought to be an invasive species, perhaps from Europe, this fungus has killed

over 6 million bats since first observed in a cave near Albany, NY in 2006 (Blehert et al. 2009). CFMR scientists, in association with researchers from the U.S. Geological Survey and several universities, are using a newly acquired collection of cultures from cave soils and affected bats to design a sensitive molecular detection technique that can be used to screen for the pathogen in the environment and in affected animals. These researchers are also identifying and cataloging closely related, nonpathogenic species of *Geomyces*, previously unknown to science that may be able to out-compete the pathogen within the cave or on bat skin. These organisms could potentially be developed as biological control agents (Lindner et al. 2010; Lorch et al. 2013; Mueller et al., 2013).

In addition to harboring wildlife, many forests today are potential sources of renewable energy. Viewing forests as a source of bioenergy is becoming a vital component of forest management, but this type of management necessitates the removal of large amounts of biomass, either for burning directly or for the production of highly valued biofuels . Unfortunately, the long-term effects of removing large amounts of biomass on forest health and forest sustainability are unknown. Woody biomass is broken down by fungi to create forest soil. The removal of large amounts of woody debris may result in nutrient deficiencies and losses in forest productivity. In addition, many wood-inhabiting fungi are considered endangered species and their presence and distribution can be used as an indicator of forest health. CFMR scientists are collaborating with university researchers on studies currently in place in the Upper Midwest and Rocky Mountain region to monitor fungal distribution changes over time under different management regimes with the goal of creating sustainable management strategies for biomass harvesting (Brazee et al. 2012). Previous studies have shown that statistically rigorous sampling of the long-lasting, wood- inhabiting fungi is an excellent tool for assessing fungal diversity and for evaluating forest management strategies and forest health (Lindner et al. 2006). Basic studies in fungal biosystematics conducted by CFMR scientists in which fungal species are defined and described are essential as identification tools needed for this type of assessment (Burdall 1985; Burdall and Banik 2001; Larsen and Cobb-Pouille 1990; Nakasone and Burdall 1995; Nakasone 1997; Ortiz-Santana et al. 2007).

Fungi also have commercial value as sources of food and medicinal compounds; many human cultures throughout the world have used fungi to improve human health for centuries. Secondary metabolites and polysaccharides from fungi display a wide range of biological activities, including antimicrobial activity against fungi, bacteria, and viruses. Many polyporoid wood decay fungi are known to have anti-inflammatory and antioxidant properties, produce compounds that can reduce the growth of cancerous tumors and stimulate the immune system, and have positive effects on cardiovascular health (Zjawiony 2004). Hundreds of cultures from the collection have been screened by pharmaceutical companies for potentially beneficial medicinal compounds that can be used to treat human diseases. CFMR scientists have also conducted collaborative research with Merck to determine factors that control microfungus diversity in tropical forests and have evaluated which of these fungi might be useful for production of pharmaceuticals (Polishook et al. 1996). CFMR personnel are currently working with researchers in the Department of Pharmacy of Concordia University Wisconsin, Milwaukee, WI, to identify cultures with potential biomedical properties. In addition to their health benefits, some fungi produce powerful toxins; mistaken identifications can be deadly. Commercial mushroom producers, as well as professional and amateur mushroom gatherers, often consult CFMR personnel for advice on fungal propagation and identification. Poison control centers and emergency room doctors contact CFMR researchers, sometimes in the middle of the night, when presented with potential poisonings.

Decayed and diseased trees are a source of danger and liability to homeowners, cities, National Parks, and National Forests. Arborists, foresters, and park personnel are being trained to identify fungi associated with hazard trees. Such assessments are needed to evaluate whether suspected trees can be left in place or need to be removed in order to protect human life and property (Glaeser and Smith 2010). Should a tree be removed if a certain type of fruiting body appears at its base or on its trunk? Does the fruiting body indicate of severe internal decay, suggestive of imminent failure, or a minor sapwood decay associated with wounding that the tree will be able to compartmentalize and remain otherwise healthy? Understanding decay patterns associated with fires and insect damage is also

important for forest managers to plan forest restoration activities. Identifications based on culture collection and herbarium reference standards have been used to assess decay patterns in fire-killed western larch (Jackson and Bulaon 2004; Jackson and Bulaon 2005), Engelmann spruce and subalpine fir (Worrall and Nakasone 2009), and beetle mortality in Lutz spruce (Glaeser et al. 2009). The identity and roles of the fungi associated with and vectored by the walnut twig beetle in the development of Thousand Canker Disease of black walnut are also being assessed.

Climate models show that the composition of North American forests will change due to the influence of climate change. Forest managers are using these projections to anticipate future forest health issues and what future North American forests will look like. The impact of climate change on the activities of wood decay fungi has been assessed by CFMR researchers (Kliejunas et al. 2009) and suggests that decay rates will probably decrease as water becomes more limiting. This can lead to increased damage from fire as decomposition rates slow and the amount of flammable slash and woody debris increases on the forest floor. In areas where moisture is not limiting, increased temperatures will likely increase decay rates, resulting in less accumulation of fuel. Decomposition of wood by brown-rot fungi, which degrade the cellulose and hemicellulose of wood cell walls but leave behind the carbon-rich lignin, sequester carbon on the forest floor and in mineral soils. Brown rotted logs dominate the organic layer in northern conifer forests, which is critical for maintaining soil fertility and forest productivity (Jurgensen et al. 1997). In interior northwestern forests, brown rotted logs are critical for forest regeneration in clearcuts, serving as nurse logs for seedlings because they retain moisture and preserve ectomycorrhizal fungal inoculum (Harvey et al. 1980; Jurgensen et al. 1997). As the lignin is slowly converted into humic acids, it augments mineral-associated carbon in forest soils. Northern conifer forests may eventually be replaced by hardwoods in response to climate change; this may reduce the input of conifer-associated brown rotted wood and thus decrease the amount of carbon sequestration, lowering soil fertility and forest productivity. CFMR has provided cultures of decay fungi to a forest restoration company in Colorado to test for accelerated decomposition of woody debris in the forest to decrease fire hazard and sequester forest

carbon. Evolutionary studies of the largest group of brown-rot fungi, known as the *Antrodia* clade, are also in progress.

Guarding the Nation's forests against potentially invasive species is one of the most important aspects of Forest Service research in which CFMR plays a critical role. In the past, devastating diseases, such as Dutch elm disease and chestnut blight, have decimated North American forests and landscapes, causing massive mortality of some of the most beloved tree species. CFMR scientists are involved in preventing the introduction of potentially invasive pathogens through the production of Pest Risk Assessments resulting from their participation in the Wood Importation and Pest Risk Assessment Mitigation Team (WIPRAMET). Insect and fungal risks that have been evaluated include the proposed import to the U.S. of logs from Siberia (USDA FS 1991), Chile (USDA FS 1993), New Zealand (USDA FS 1992) and Mexico (Tkacz et al. 1998), and of both logs and wood chips from Australia (Kliejunas et al. 2003; Kliejuanas et al. 2006) and South America (Kliejunas et al. 2001). WIPRAMET is currently producing a risk assessment that will evaluate the risk to native Hawaiian tree species from insects and fungal pathogens. Several different pathways are being assessed, including the import of ornamentals, "hitchhikers," and various forest products to the islands. CFMR was directly involved in analyzing fungi from wood chips imported from Chile. The vast majority of these fungi were harmless molds that can grow rapidly on wood and out-compete the growth of any potential pathogens, although some sapstain fungi were detected (Glaeser and Burdsall 2008). Forest pathogens occurring in subtropical forests that could invade the U.S. in response to climate change have also been identified (Banik et al. 2012; Lodge et al. 2010).

CFMR collections serve the public in more indirect ways by providing source material to scientists throughout the world as they unravel the mysteries of Kingdom Fungi. In a typical year, CFMR sends 700 – 800 fungal cultures to research laboratories in North America, Europe, Asia, and Latin and South America. Between 1999–2009, specimens, cultures and DNA sequences from CFMR collections resulted in over 2,602 citations in scientific journals (http://www.nrs.fs.fed.us/units/foresthealth/local-resources/docs/CFMR_spec_cult_cit.xls). This is a very conservative

estimate since much of the material is used without proper accreditation. Over 100 CFMR cultures were sequenced in a recent publication on the evolution of white-rot fungi in the genus *Trametes*; this study resulted in a major redefinition of this important genus (Justo and Hibbett 2011). This study is one in a series of National Science Foundation-funded projects on the evolution of white- and brown-rot fungi that are using CFMR cultures as a major source of material (Hibbett and Justo 2012). The application of DNA sequences obtained from CFMR cultures was also used in a widely publicized study published in *Science* to explore the evolution of enzymatic lignin decomposition. The authors concluded that the evolution of white-rot fungi corresponded with the sharp decrease in the rate of coal formation at the end of the Carboniferous period (Floudas et al. 2012). The improved understanding of lignin degradation mechanisms gained through the sequencing of CFMR cultures will aid industrial microbiologists in developing more efficient processes for converting woody biomass into biofuels.

A new project related to development of industrial enzymes, including those used in biofuel processing and discovery of new pharmaceuticals, is the "1000 Fungal Genome" program, a Department of Energy-based initiative to develop total genome sequences of 1000 different fungi distributed throughout the fungal kingdom (Spatafora 2011). This effort will serve as a basis for future work in fungal genomics, physiology, and biochemistry and will lead to advances in medicine and bio-based industries as researchers unravel the mechanisms behind disease development and the production of fungal secondary metabolites, such as antibiotics. Cultures from the CFMR collection are particularly valued in this effort since many of them are in a haploid condition (N), containing only one copy of fungal DNA per cell instead of the more typical 2N or N+N state. The use of haploid cultures greatly facilitates data analyses and simplifies this complex research effort. CFMR scientists are also participating in the initial production of a proposed North American *Mycoflora* (Bruns 2011), a massive "wiki"-like on-line encyclopedia that will combine molecular data with other traditional identification tools – keys, pictures, ecological and morphological descriptions - to become a centralized location of fungal information for all macrofungi in North America. The CFMR collections are also included as a resource in the U.S. Culture

Collection Network (McCluskey 2012) – a 5-year National Science Foundation- funded project that will network all U.S. plant-associated microbial collections and develop standardized protocols for their management.

The CFMR collections are an important asset to science, the Forest Service, and the American public. The use and value of the collections have changed and increased since its establishment in the 1920s. These collections are repositories for the future, preserving the genetic heritage of the past and present, and need to be preserved. More information about the collections and a link to the searchable database can be found at <http://www.fpl.fs.fed.us/research/centers/mycology/culture-collection.shtml>.

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PATHOGEN AND BARK BEETLE RESPONSES TO FOREST MANAGEMENT IN THE SOUTHERN SIERRA NEVADA

Ashley E. Hawkins¹ and David M. Rizzo¹

ABSTRACT

Increasing temperatures have been implicated in recent widespread pathogen and bark beetle driven tree mortality in western North America (Woods 2005; Kurz et al. 2008; van Mantgem 2009). Pathogens and bark beetles can be the most important agents of tree mortality in natural forest systems of western North America. Despite their importance it is unclear how pathogens and bark beetles interact with common forest management practices to drive changes in ecosystem processes, ecological dynamics, and carbon cycling.

Logging and fire suppression activities over the past century have significantly altered the structure and composition of forests in western North America. Fire suppression has increased stand densities, reduced densities of fire tolerant/shade intolerant species, and reduced the number of large-diameter trees, essentially converting forests to even-aged, low-diversity plantations (Odion et al. 2004; McKelvey et al. 1996). These changes have negative impacts on wildlife and plant communities and predispose forests to high-intensity wildfire (North et al. 2009). In addition, large-scale tree mortality can cause forests to transition from carbon sinks to sources of atmospheric carbon (Kurz et al. 2008).

In the Sierra Nevada forest managers commonly apply thinning and burning treatments to restore forest structure and composition but it is unclear how these treatments affect ecosystem process and ecological dynamics. To address this issue the Teakettle Ecosystem Experiment was established in 1998 on the Teakettle Experimental Forest, Sierra National Forest, California. Two burning treatments (burn, no-burn) were crossed with three thinning treatments (overstory thin, understory thin, no-thin) yielding six total treatments. Treatments were applied to 18, four-hectare plots so that each treatment was replicated three times.

DATA COLLECTION

All trees in the experimental plots were mapped with a surveyor's total station and breast-height diameter measurements were taken prior to treatments. All trees were re-measured at breast-height in 2004 and in 2011. Regularly spaced sampling points (10 m intervals in one plot per treatment and 25 m intervals in remaining plots) were established to facilitate spatial sampling of seedlings, woody biomass accumulation, and soil moisture. Pathogen and bark beetle data were collected on all trees (> 30,000) prior to treatments and in 2004-2005 to determine pre- and post-treatment levels of pathogens and insects and effects on tree mortality (Smith et al 2005; Maloney et al 2008). I have re-collected pathogen and bark beetle data on 14 plots and will complete the fieldwork over the summer of 2013. I

am collecting data on all major pathogens and bark beetles that cause or contribute to tree mortality. Identification of pathogens and bark beetles is based on observations of signs and symptoms. Standard DNA isolation and PCR methods are used when signs and symptoms are unclear.

QUESTIONS

- What are the effects of treatments on the level of pathogens, bark beetles, and tree mortality a decade after treatment application?
- What are the effects of pathogens and bark beetles on tree mortality, tree community composition, spatial distribution, and size distribution?
- What is the contribution of different pathogens and bark beetles to woody debris deposition and carbon cycling?
- How do pathogens, bark beetles, fire, treatments, competition, and the environment interact to drive tree mortality?

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- Are pathogens and bark beetles driving tree population growth rates?

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VITALITY AND DEFENSE MECHANISMS IN BARK-STRIPPED CEDAR TREES

Paul Hennon¹ and Nancy Turner²

Native People along the Pacific Coast have collected bark from cedar trees for millennia (Turner et al. 2009). How do these culturally modified cedar trees appear so healthy, and live so long, after having portions of their bark removed? This poster reviews studies on heartwood chemistry, deterioration in dead trees, and injuries caused by brown bears to build a scenario that explains how static and active mechanisms in cedar trees compartmentalize wood decay and allow bark-stripped trees to survive for centuries.

Heartwood chemistry is a key defense: The heartwood of cedars contains compounds that protect against fungal wood decay (Kelsey et al. 2005). Sapwood lacks these chemicals and it decays readily when dead (Hennon et al. 2000). What can we learn from bears? Brown bears bark-strip yellow-cedars every spring in some forests of Alaska. The response by cedar trees is the same, whether bears or people remove the bark. From dissections of bear-wounded cedar trees (Hennon et al. 1990), we learned that decay is limited to wood that was sapwood directly beneath the wound at the time of injury.

Bark removal provides an infection court for fungi to invade dead wood cells in the exposed injury. Sapwood begins to decay, but these fungi cannot spread to heartwood due to its phenolic defensive compounds. Concentrated heartwood compounds actively form in live sapwood directly adjacent to dead sapwood to protect against circumferential fungal spread. A suberized layer of cells is produced by the cambium which blocks the spread of fungi to any new wood produced after the wounding event. Radial growth rate near the injury (callus) is greater than typical annual ring growth. This helps stabilize the tree and grow wood tissue over the injury. Sapwood killed at the time of injury becomes fully decayed by fungi within two decades (Hennon et al. 2000).

Callus growth continues. Decayed sapwood sloughs away leaving a thin discolored band of wood which appears fissured on the surface. Long-term decay of exposed heartwood is faster at the bottom of the wound where it is in contact with the ground (Hennon et al. 2007), otherwise the old injury experiences little change.

CONCLUSIONS

Cedars have defenses that are both passive (existing heartwood) and active responses (production of suberized layers, heartwood compounds in live sapwood, and callus growth). Many culturally modified trees are found on productive soils because this is where people find large trees free of lower branches growing. The long term viability of culturally modified trees may be explained by several defense mechanisms of trees growing on these favorable sites in a maritime climate and the custom of never removing bark on too much of the tree's circumference (Turner et al. 2009).

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CHEMICAL TREATMENT TO SANITIZE *PHYTOPHTHORA RAMORUM* COLONIZED TIMBER PRODUCTS

Joey Hulbert^{1,2}, Jeff Morrell¹, and Everett Hansen²

ABSTRACT

The discovery of *Phytophthora ramorum* in Oregon in 2001 led the State to establish a strictly controlled quarantine area. Since then, the quarantine has expanded several times to incorporate newly discovered infested areas. Continued expansion will lead to an ever increasing area that restricts timber harvest. Developing methods for mitigating the risk of spreading the pathogen on logs from infested areas could maximize utilization. The objective of this study was to develop preliminary data on the fungitoxicity of boron to *P. ramorum* under laboratory conditions. Disodium octaborate tetrahydrate (DOT) was added to corn meal agar at levels designed to produce 0.1, 0.5, 1.0, 3.0 or 5.0 percent boric acid equivalent (BAE) concentrations. Agar plugs cut from the edges of actively growing cultures of *P. ramorum* (NA-1 Isolate number: 4313, 7904, & 9488) were placed on petri dishes containing the borate amended agar. Plates were incubated for 13 days at room temperature and growth was measured (Table 1). Plugs where *P. ramorum* failed to grow were removed and placed on non-boron amended corn meal agar to determine if the effect was fungistatic or fungicidal. Plates with original plugs were analyzed 6 days after re-subbing onto non-boron amended corn meal agar. The three isolates were capable of limited growth on media containing 0.1 percent BAE DOT; media containing 0.5 percent BAE or more of DOT completely inhibited growth of *P. ramorum* (Figure 1). The 0.1 percent BAE DOT concentration reduced average radial growth for isolates 4313, 7904, and 9488 by 89.6, 92.9, and 89.5 percent, respectively, compared to the control (Table 1). The original inoculum plugs from the 3 percent and 5 percent BAE DOT concentrations failed to grow after six days of incubation on non-boron amended corn meal agar for all isolates (Table 2). Although growth was observed after six days on the lower concentrations, extended incubation periods are necessary to determine whether the 3 percent and 5 percent concentration are in fact fungicidal. This work was preliminary, but indicates that boron has potential for arresting *P. ramorum* growth.

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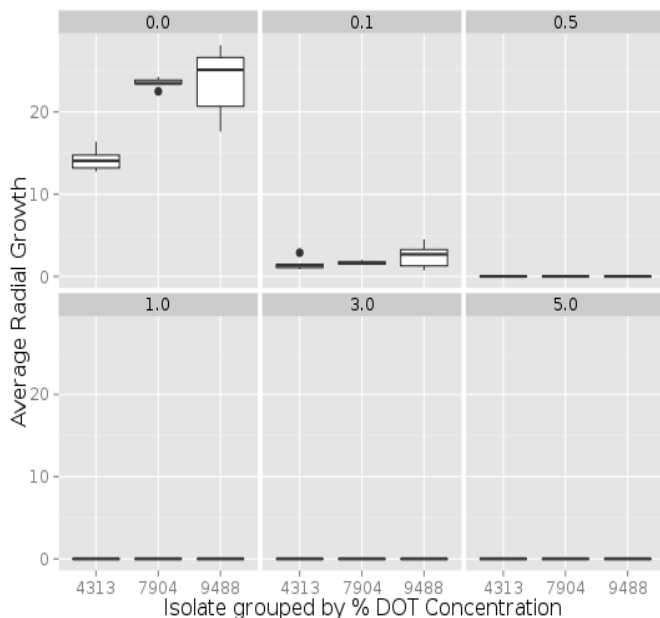


Figure 1. Box plots for average radial growth, grouped by isolate and concentration.

Table 1. Average radial growth after 13 days for each isolate and concentration.

Isolate	DOT Concentration	Average Radial growth (mm)
4313	0.0	14.19
	0.1	1.48
	0.5	0.00
	1.0	0.00
	3.0	0.00
	5.0	0.00
9488	0.0	23.64
	0.1	2.48
	0.5	0.00
	1.0	0.00
	3.0	0.00
	5.0	0.00
7904	0.0	23.55
	0.1	1.68
	0.5	0.00
	1.0	0.00
	3.0	0.00
	5.0	0.00

Table 2. Concentration summaries for each Isolate.

Isolate	Concentration	Fungistatic	Fungicidal*
4313	0.0	-	-
	0.1	-	-
	0.5	+	-
	1.0	+	-
	3.0	+	+
	5.0	+	+
9488	0.0	-	-
	0.1	-	-
	0.5	+	-
	1.0	+	-
	3.0	+	+
	5.0	+	+
7904	0.0	-	-
	0.1	-	-
	0.5	+	-
	1.0	+	-
	3.0	+	+
	5.0	+	+

*Fungicidal diagnosis was completed under a limited amount of time and is subject to change.



SUDDEN OAK DEATH ERADICATION IN SOUTHWEST OREGON: 2001-2012

Alan Kanaskie¹, Everett Hansen², Ellen Michaels Goheen³, Nancy Osterbauer⁴, Michael McWilliams¹, Jon Laine¹, Michael Thompson¹, Stacy Savona¹, Bill Woosley¹, Wendy Sutton², Paul Reeser², Rick Schultz⁵, and Dan Hilburn⁴

ABSTRACT

Sudden Oak Death, caused by *Phytophthora ramorum*, is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens this species throughout its range in Oregon. The disease was first discovered in coastal southwest Oregon forests in July 2001. An interagency team attempted to eradicate the pathogen through a program of early detection surveys followed by destruction of infected and nearby host plants. Cutting and burning host plants eliminated the pathogen from an estimated 50 percent of infested sites. Large treatment areas (150 to 200m buffer around infected trees) were more effective at slowing disease spread than smaller treatment areas.

Despite the eradication effort, the disease continued to spread slowly in a predominantly northward direction. During the 11-year period, the disease spread from the initial infestations southward 1.9 km, and northward and eastward 27.9 km and 12.8 km, respectively (Figure 1). Continued spread of sudden oak death is attributed to the slow development of symptoms in infected trees which hinders early detection, and to delays in completing eradication treatments which allow disease spread from known infestations.

A marked increase in disease in 2010 and 2011 indicated that eradication costs on private lands would exceed available or expected funds. In early 2012 the Oregon State quarantine regulations were revised to reflect the financial reality of managing sudden oak death. The key provisions of the new quarantine are:

1. Establishes a “generally-infested area” within the quarantine boundary where *P. ramorum* has been commonly found and complete eradication of the pathogen is impossible or impractical. Within the generally infested area eradication treatments are no longer required by the State.
2. Defines two types of infested sites based on their importance for spread of disease:
 - A. Type 1 sites are infested sites considered to be of highest risk for spread of *P. ramorum* into previously un-infested areas. They typically are located outside of the generally infested area. The highest priority sites are those closest to or

beyond the existing quarantine boundary. Eradication treatments are required. Cost of treatment will be borne by the State if funds are available.

- B. Type 2 sites are infested sites considered to be of less risk for spread of *P. ramorum* into previously un-infested areas. Type 2 sites typically are located inside of the generally infested area. Eradication treatments are not required, but disease suppression through best management practices is encouraged.
3. Allows increased utilization of tanoak within the quarantine area:
 - A. Inside the generally infested area tanoak may be used as non-commercial firewood, but it cannot leave the generally infested area.
 - B. Outside of the generally infested area tanoak cannot leave an infested site or eradication treatment area, but it can be transported out of the quarantine area if from a “disease free area”, which is defined as an area located more than 1/4 mile from the generally infested area or any other infested site, and which has been officially surveyed within the past 6-months and found free of *P. ramorum*.

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The initial goal of complete eradication in Curry County forests is now considered unachievable. The goal now is to slow further disease spread by: 1) early detection and rapid eradication of new infestations that are epidemiologically important; 2)

reducing inoculum levels wherever practical through cost-share projects and best management practices, and; 3) improved education and outreach to prevent spread by humans.

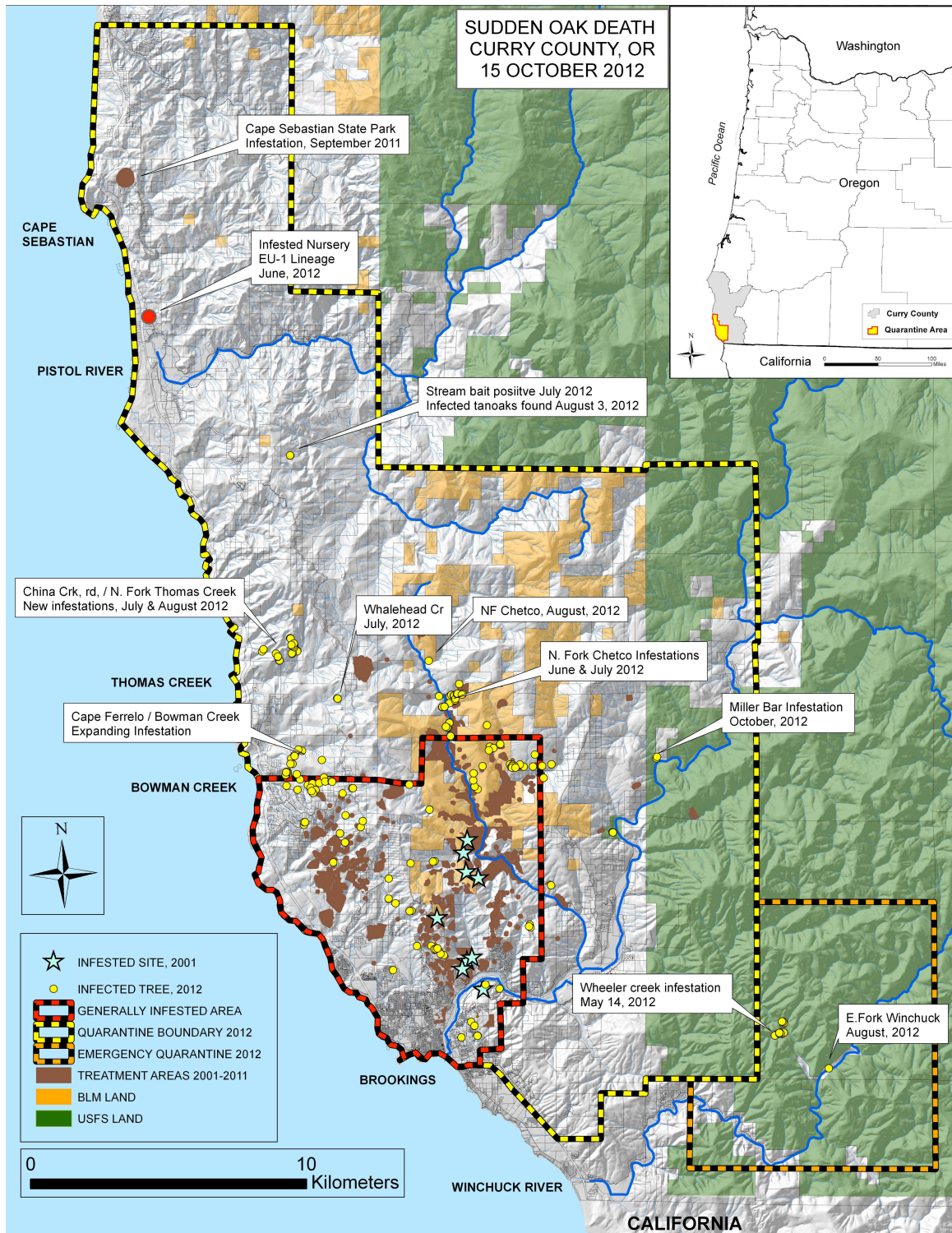


Figure 1. Location of sites infested with *Phytophthora ramorum* in southwest Oregon discovered in 2012 (as of October 1, 2012). Text boxes highlight areas of significant sudden oak death activity in 2012. Sites enlarged for visibility.



CYLINDROCARPON SPP. IN DOUGLAS-FIR FOREST NURSERY IN THE PNW USA AND THEIR MANAGEMENT

Mahsa Khorasani¹, Nathan Johnson¹, and Robert Edmonds¹

INTRODUCTION

Cylindrocarpon spp. cause soil-borne diseases in Douglas-fir bare root nurseries in the Pacific Northwest U.S. (Figure 1). These soil-borne diseases are difficult to control and have typically been controlled by fumigating nursery bed soils with methyl bromide. Newer integrated pest management approaches have been successful to some degree in reducing soil disease populations in combination with fumigation, and include cover crop manipulation, bare-fallow rotation, tillage, improved drainage, and improved seedling grading. However, some of these pathogens (specifically *Cylindrocarpon* spp.) can survive even when treated with methyl bromide. Methyl bromide is about to be phased out because of its negative environmental effects.

Biocontrol agents have some promise for controlling *Cylindrocarpon*. Commercial biocontrol agents (such as Cease – *Bacillus subtilis*, Actinovate – *Streptomyces lydicus*, Soil Guard – *Gliocladium virens*, and Root Shield – *Trichoderma harzianum*) are available, but they have been only sporadically tested. Knowledge of the relative efficacy of biocontrol agents could decrease fungicide use and reduce fears of fungicide tolerance buildup, while improving seedling yield and performance. Furthermore, compared to other pathogens there is not much known about *Cylindrocarpon* spp. and their pathogenicity.

Another biocontrol approach involves the use of mycoviruses. Mycoviruses infect fungi and have the potential to control fungal diseases of crops when associated with fungus hypovirulence. Typically mycoviruses have double-stranded (ds) or single-stranded (ss) RNA.

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Figure 1. Douglas-fir seedling stunting caused by *Cylindrocarpon* species.

OBJECTIVES

The objectives of this research were to determine: (1) the species of *Cylindrocarpon* present in nursery soils, (2) if commercial biocontrol agents can be used against *Cylindrocarpon* spp., and (3) existence of mycoviruses in *Cylindrocarpon*.

METHODS

Cylindrocarpon isolates were obtained from three Douglas-fir nurseries in WA and OR and grown on PDA. DNA was obtained from mycelial scrapings, PCR was conducted, and cleaned PCR products were sequenced. The biological control agents Cease, Actinovate and Soil Guard were grown in culture with *Cylindrocarpon* and growth inhibition was measured. The presence of mycoviruses was to be determined by isolation of the total RNA from mycelia using a Qiagen kit, and digesting the DNA and ssRNA by using DNase I and S1 nuclease enzymes. Unfortunately, this method did not work well, so other techniques were used such as:

- Extraction of total RNA with Trizol instead of Qiagen.
- Extraction of dsRNA with phenolic acid.

RESULTS AND DISCUSSION

There is a lot of variation in appearance of *Cylindrocarpon* in culture (Figure 2). Three different *Cylindrocarpon* spp. were identified (*C. destructans*, *C. liriodendri* and *C. pauciseptatum*). Overall in the three nurseries *C. destructans* was dominant (59.0 percent), compared to 38.5 percent for *C. liriodendri* and only 2.5 percent for *C. pauciseptatum*, which was only detected at one of the nurseries.



Figure 2. Variation in appearance of *Cylindrocarpon* isolates growing in culture.

The biological control agents Cease, Actinovate, and Soil Guard were not very effective at inhibiting the

growth of any of the *Cylindrocarpon* spp in culture (Figure 3) Cease was better than Actinovate which was better than Soil Guard, but the highest growth inhibition was only 47.6 percent. There was no difference in inhibition among the three *Cylindrocarpon* species.

We have not yet found evidence for the presence of mycoviruses in *Cylindrocarpon* cultures. But using some real dsRNA which was digested with S1 nuclease we were not convinced that there is no virus in the fungal samples. The Cf11 chromatography method will be tried in the future.

CONCLUSIONS

- Three different *Cylindrocarpon* spp. were identified: (*Cylindrocarpon destructans*, *Cylindrocarpon liriodendri*, and *Cylindrocarpon pauciseptatum*).
- The biological control agents Cease, Actinovate, and Soil Guard were not very effective at inhibiting the growth of any of the *Cylindrocarpon* spp. in culture.
- We have not yet found evidence for the presence of mycoviruses in the *Cylindrocarpon* cultures.

This research was funded by the USDA, Washington State Pesticide Council, and Weyerhaeuser.

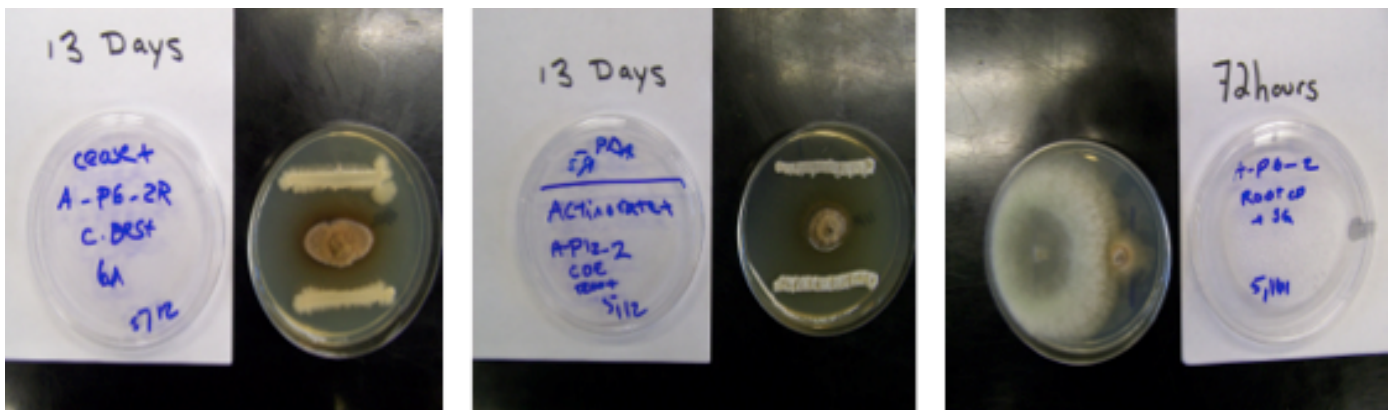


Figure 3. Small growth inhibition of *C. destructans* by Cease (left), Actinovate (middle), and Soil Guard (right).



ARMILLARIA PHYLOGENY BASED ON *tef-1α* SEQUENCES SUGGESTS ONGOING DIVERGENT SPECIATION WITHIN THE BOREAL FLORISTIC KINGDOM

Ned B. Klopfenstein¹, John W. Hanna¹, Amy L. Ross-Davis¹, Jane E. Stewart², Yuko Ota³, Rosario Medel-Ortiz⁴, Miguel Armando López-Ramírez⁴, Rubén Damián Elías-Román⁵, Dionicio Alvarado-Rosales⁵, and Mee-Sook Kim⁶

INTRODUCTION

Armillaria plays diverse ecological roles in forests worldwide, which has inspired interest in understanding phylogenetic relationships within and among species of this genus. Previous rDNA sequence-based phylogenetic analyses of *Armillaria* have shown general relationships among widely divergent taxa, but rDNA sequences were not reliable for separating closely related North American species, such as *A. gallica*, *A. calvescens*, *A. cepistipes*, and *A. sinapina*, or other closely related Eurasian species (Kim et al. 2006). Recent studies have shown that *translation elongation factor 1-α* (*tef-1α*) sequences appear quite useful for phylogenetic analysis of *Armillaria* spp. from diverse global regions (Maphosa et al. 2006; Antonín et al. 2009; Hasegawa et al. 2010; Ota et al. 2011; Brazeo et al. 2011; Mulholland et al. 2012; Ross-Davis et al. 2012; Tsykun et al. 2012). The objective of this study is to determine phylogenetic relationships among northern hemisphere *Armillaria* spp. based on available *tef-1α* sequences from well-characterized isolates.

MATERIALS AND METHODS

The *tef-1α* sequences used in this study were reported by Maphosa et al. (2006), Antonin et al. (2009), Hasegawa et al. (2010), Ota et al. (2011), Brazeo et al. (2011), Mulholland et al. (2012), Ross-Davis et al. (2012), and Tsykun et al. (2012). In addition, *tef-1α*

sequences from GenBank and from *Armillaria* isolates from Mexico and Eurasia were included in the phylogenetic analyses. The *tef-1α* sequences of *Pleurotus pulmonarius* (D480: EU204111) and *Tricholoma myomyces* (KMS589: DQ367429) were obtained from GenBank to serve as outgroups. A phylogenetic network analysis was implemented using SplitsTree (Huson and Bryant 2006) (Figure 1). Additional phylogenetic analyses are being conducted using maximum likelihood and Bayesian methods (data not shown).

RESULTS AND DISCUSSION

According to the preliminary *tef-1α*-based phylogeny from this study (Figure 1), *Armillaria* spp. from the Boreal Floristic Kingdom are distributed among several major clades, including 1) *A. solidipes/ostoyae* clade (North American *A. solidipes/ostoyae*, *A. gemina*, and *A. sinapina*; and Eurasian *A. borealis* groups, *A. solidipes/ostoyae*, *A. cepistipes*, and *A. sinapina*); 2) *A. gallica* clade (North American *A. gallica* groups, *A. calvescens*, *A. cepistipes*, *A. nabsnona*, and *A. altimontana*; Japanese *A. nabsnona* and Nag E; Asian *A. gallica*; and European *A. gallica*); 3) *A. mellea* clade (North American *A. mellea* groups; European *A. mellea*; and Japanese *A. mellea*); and 4) exannulate *Armillaria* clade (Eurasian *A. ectypa* and *A. socialis/tabescens* groups; and North American *A. tabescens*). A separate, fifth, clade comprises an undescribed *Armillaria* species from Mexico, but further work is needed to characterize members of this clade.

These results provide preliminary evidence that 1) currently recognized *A. mellea* likely comprises multiple species; 2) currently recognized *A. gallica* likely comprises multiple species; 3) currently recognized *A. borealis* apparently comprises at least two distinct species; 4) Eurasian *A. cepistipes* and North American *A. cepistipes* reside in distinct major clades and likely represent very distinct species; 5) North

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American *A. solidipes/ostoyae* is genetically distinct from Eurasian *A. solidipes/ostoyae*; 6) North American *A. socialis/tabescens* appears phylogenetically distinct from Eurasian *A. socialis/tabescens*; 7) a well-separated clade comprises *Armillaria* isolates from Mexico that

represent an undescribed species (Elias-Roman et al. this proceedings); and 8) the genus *Armillaria* likely comprises multiple other cryptic species. Continued phylogenetic studies are needed to confirm genetic relationships within the *Armillaria* genus.

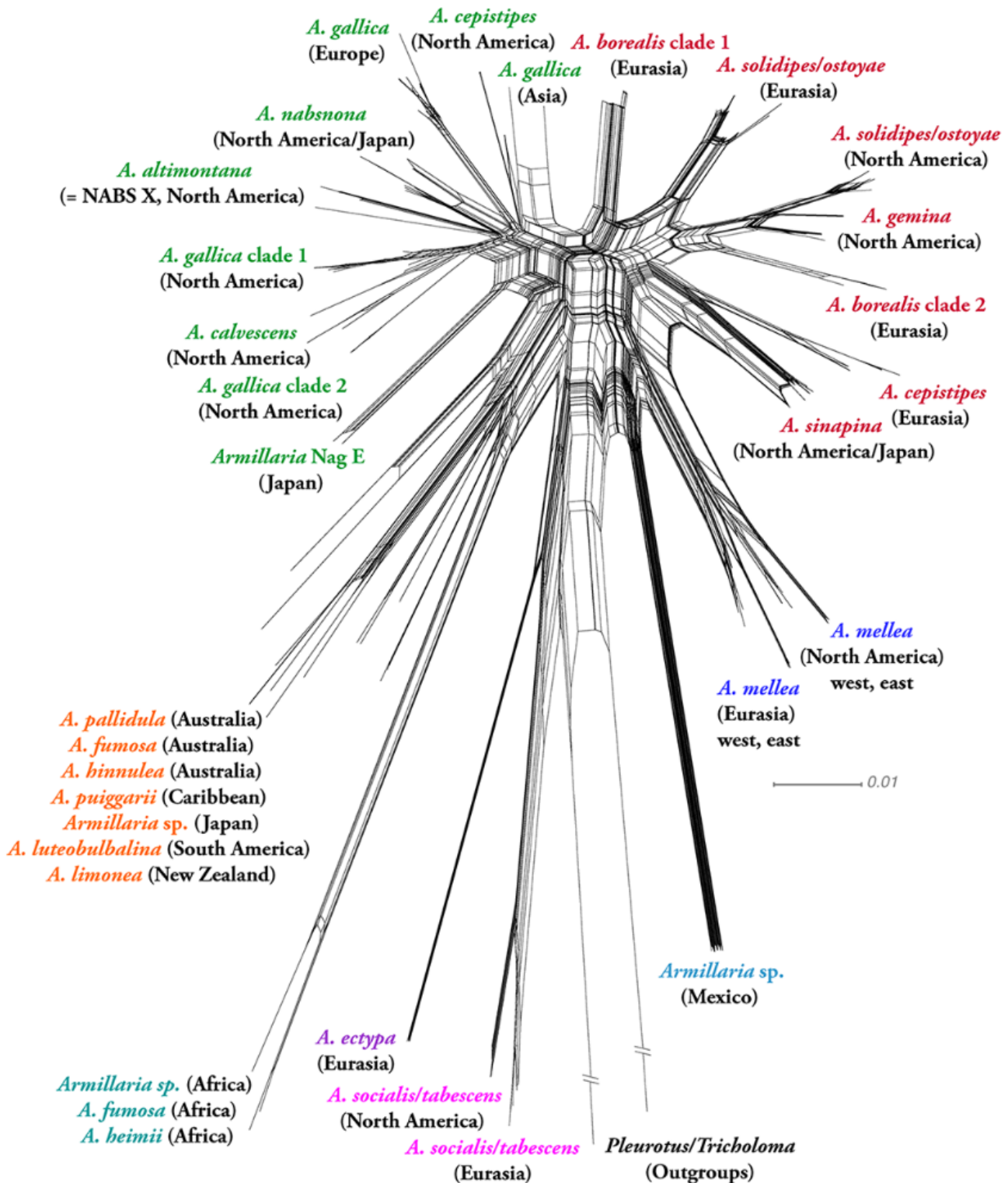


Figure 1. SplitsTree phylogenetic network of global *Armillaria* spp., based on translation elongation factor 1- α .

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DNA-BASED IDENTIFICATION OF *ARMILLARIA* ISOLATES FROM PEACH [*PRUNUS PERSICA* (L.) BATSCH] ORCHARDS IN MÉXICO STATE, MÉXICO

Rubén D. Elías-Román^{1,5}, Ned B. Klopfenstein², Mee-Sook Kim³, Dionicio Alvarado-Rosales¹, John W. Hanna², Amy L. Ross-Davis^{2,4}, Remigio Anastacio Guzmán-Plazola¹, Guillermo Calderón-Zavala¹, and Antonio Mora-Aguilera¹

ABSTRACT

A collaborative project between the Programa de Fitopatología, Colegio de Postgraduados, Texcoco, Edo. de México and the USDA Forest Service-RMRS, Moscow Forest Pathology Laboratory began in 2011 to identify which species of *Armillaria* are causing widespread and severe damage to the peach orchards from México State, México. We are employing a DNA-based approach in which the intergenic spacer 1 region (IGS1) of nuclear rDNA and the translation elongation factor-1 alpha gene will be sequenced and compared to known *Armillaria* species to facilitate species identification.

INTRODUCTION

Peach cultivation constitutes an important crop in México, with a production area of ca. 45,000 ha. Nearly 65 percent of the national production occurs in the highlands of central México (Michoacan, Morelos, and México States; SIAP 2010). In these areas, peach orchards are typically established in areas that were cleared of the native forests (Figure 1). In México State, peach orchards are relatively short-lived, with a production life span of about 10 years. *Armillaria* species are considered the most damaging pathogens of peach trees in this region, where they cause significant annual mortality of orchard-grown peach trees.

Information about *Armillaria* spp. in Mexican fruit orchards is very limited; however, some general information on *Armillaria* species in México is available in reports of edible mushrooms, commercial mushrooms, and ethnobotany (Montoya et al. 2003). Specific information is also found about the identification of *Armillaria* species collected from forests of central México using known haploid tester strains from U.S. (Alvarado-Rosales and Blanchette 1994). Currently, DNA-based diagnostics have not been widely applied to identify *Armillaria* species from México.

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Figure 1. Fruit orchards established in areas where native forests are cleared.

OBJECTIVE

The objective of this project is to use DNA-based methods to identify 49 isolates of *Armillaria* collected from peach trees growing in orchards throughout México State for comparisons with *Armillaria* species from native forests. This information will be used to document the distribution of *Armillaria* spp. and help develop species-specific *Armillaria*-resistant peach rootstocks.

METHODS

The *Armillaria* isolates (n = 49) were collected from 15 peach orchards throughout México State. For each of these isolates the intergenic spacer 1 (IGS1) region of rDNA was sequenced, and the translation elongation factor 1 alpha (*tef-1 α*) was sequenced for 24 isolates.

The protocol of Kim et al. (2006) was used for PCR amplification of IGS1. Template DNA used for *tef-1α* PCR was obtained by the protocol of Zhang et al. (2010). PCR was performed using the methods of Ross-Davis et al. (2012) except that primers were replaced by ARMEFF (5 ft. CGT GAY TTY ATC AAG AAC ATG AT 3 ft.) and ARMEFR (5 ft. TAC CCG TTC GGC GAT CAA TCT 3 ft.) designed by J.W. Hanna (USDA Forest Service, RMRS). PCR products were sequenced on an ABI3730xl DNA Analyzer (Applied Biosystems,

Foster City, CA, U.S.) at the University of Wisconsin Biotechnology Center (Madison, WI, U.S.). The sequences were ed. with BioEdit (ver. 7.1.3; Ibis Biosciences, Inc.). For phylogenetic analysis, parsimony analyses were performed with PAUP (4.0b10) to determine phylogenetic relationships among representative *Armillaria* isolates from species found in Mexican peach orchards and the other North American *Armillaria* species based on *tef-1α* sequence.

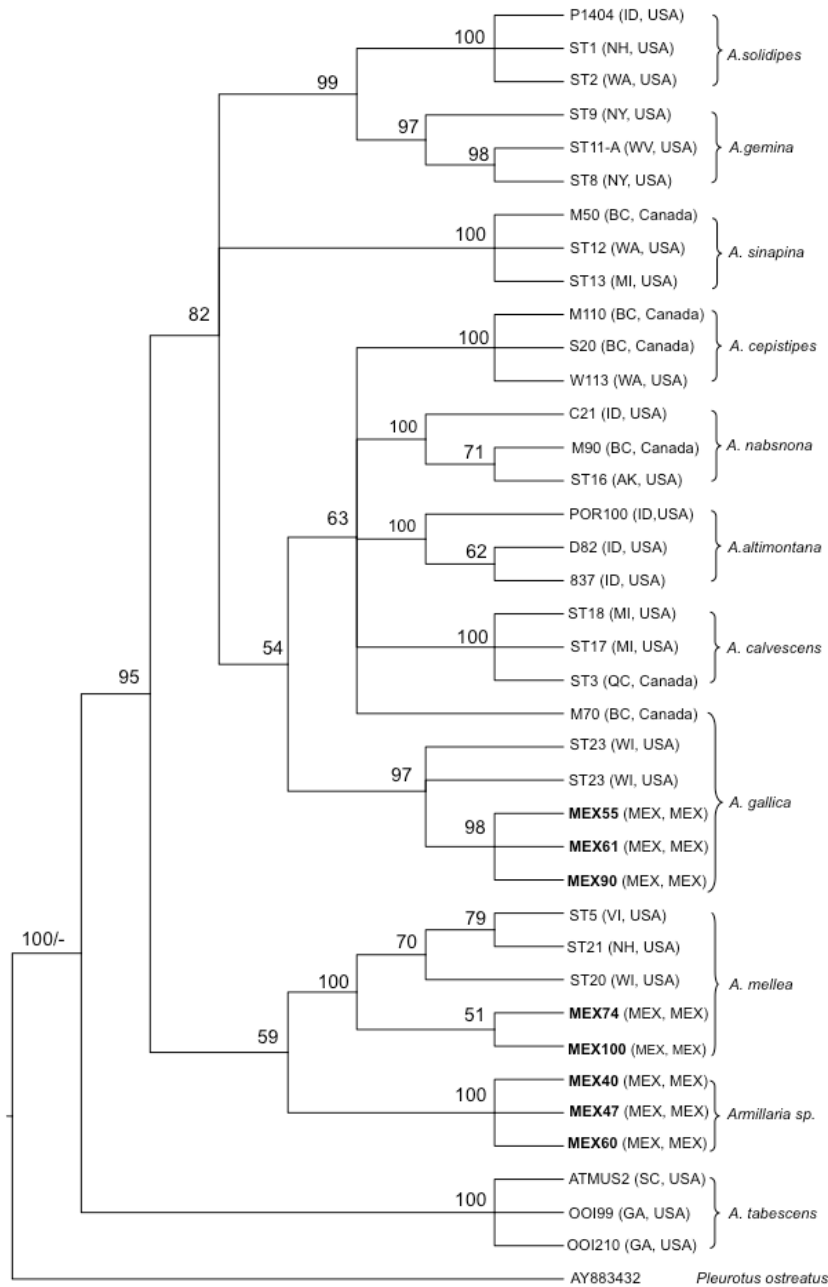


Figure 2. A 50 percent majority-rule bootstrap-consensus tree from the parsimony analysis of the translation elongation factor 1-alpha gene (*tef-1α*). Bootstrap supports are indicated above branches based on 1000 bootstrap replicates.

RESULTS AND DISCUSSION

Based on IGS1 sequences, all *Armillaria* isolates from infected peach trees could be assigned to three different taxa: Five of the 49 isolates were classified as *A. mellea*, eight isolates belonged to a single clade within the *A. gallica* complex, and the remaining 36 isolates were similar to each other, but distinct from other *Armillaria* species for which IGS1 sequences were available. Parsimony analysis of *tef-1 α* sequences revealed the presence the *A. mellea*, *A. gallica*, and a unique clade that likely represents an undescribed species (Figure 2).



Figure 3. Mushrooms (basidiomata) of an undescribed *Armillaria* species frequently isolated from infected peach trees in México State.

The undescribed *Armillaria* sp. (Figure 3) is quite distinct from species typically found in association with *Armillaria* root disease of peach trees in the southeastern U.S. (*A. tabescens*) and México (*A. gallica*). Work is underway to formally describe this undescribed *Armillaria* species. Because this undescribed *Armillaria* sp. is quite damaging to peach production, it is important to document its distribution so appropriate disease management practices can be implemented. In addition, the development of specialized rootstock is perhaps required for resistance to this undetermined *Armillaria* sp. (Schnabel et al. 2005).

Understanding the distribution of *Armillaria* species in México is also critical to predict potentially invasive *Armillaria* species for other States in México. This information also lays a foundation for predicting potential influences of climate change on *Armillaria* root disease.

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DNA-BASED APPROACHES TO IDENTIFY FOREST FUNGI IN PACIFIC ISLANDS: A PILOT STUDY

Anna E. Case¹, Sara M. Ashiglar¹, Phil G. Cannon², Ernesto P. Militante³, Edwin R. Tadiosa⁴, Mutya Quintos-Manalo³, Nelson M. Pampolina³, John W. Hanna¹, Fred E. Brooks⁵, Amy L. Ross-Davis¹, Mee-Sook Kim⁶, and Ned B. Klopfenstein¹

INTRODUCTION

DNA-based diagnostics have been successfully used to characterize diverse forest fungi (e.g., Hoff et al. 2004, Kim et al. 2006, Glaeser & Lindner 2011). DNA sequencing of the internal transcribed spacer (ITS) and large subunit (LSU) regions of nuclear ribosomal DNA (rDNA) has proved especially useful (Sonnenberg et al. 2007, Seifert 2009, Schoch et al. 2012) for identification. Most DNA-based identifications of forest fungi involve taxa that have been previously well-characterized by morphology or mating tests. However, the efficiency of DNA-based identifications of forest fungi in soils or rotting wood, especially in biodiversity hotspots like the Pacific Islands, is largely unexplored. We are conducting a preliminary study with the following objectives: 1) to determine the efficacy of DNA-based identifications of fungi associated with roots and wood rot in the Pacific Islands, from Hawaii to the Philippines; and 2) to evaluate the usefulness of sequences from nuclear rDNA regions, such as the LSU and ITS, for identifying the fungi collected in our surveys.

METHODS

Roots from nine *Pinus merkusii* and *P. kesiya* trees were collected from the forests of Luzon, Philippines (Figure 1). We surface-disinfected the roots and isolated fungal cultures on benomyl-dichloran-streptomycin (BDS) and malt-extract agar (MEA) with streptomycin.

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Sporocarps from Pohnpei, the Federated States of Micronesia (FSM), and Hawaii associated with heart, butt, or root rot were also collected and dried. DNA was extracted from the axenic cultures and dried sporocarps of each sample and PCR was conducted on both the ITS (ITS1-5.8S-ITS2) and LSU (D-domain) regions. PCR products were sequenced and compared to the GenBank[®] database (<http://www.ncbi.nlm.nih.gov/genbank/>) using BLAST[®]. As this project continues, additional fungal isolates associated with sporocarps and root-, butt- and wood-rot will be collected and sequenced from the Federated States of Micronesia, Hawaii, Guam, and other islands in the South Pacific.

PRELIMINARY RESULTS

We used DNA-based identification methods to help identify 25 fungal isolates from the roots of *P. merkusii* and *P. kesiya* collected in the Philippines, and four additional sporocarp samples from Pohnpei, FSM, and Hawaii, U.S. Comparisons of ITS and LSU sequences from the isolates and GenBank[®] sequences are summarized in Table 1. Of the isolates for which both ITS and LSU sequences were available, 38 percent (11 of 29) produced general agreement between ITS and LSU regions as to the closest species- or genus-level matches in GenBank[®] (blue and green highlighted rows in Table 1). The remaining isolates (not highlighted in Table 1) either did not show genus-level agreement between their ITS and LSU regions, or sequence data were lacking for one of the regions. Therefore, 62 percent (18 of 29) of these isolates may share ITS or LSU similarities with analogous sequences from identified genera or species in GenBank[®], but they cannot yet be definitively assigned to a taxon. The diverse genera identified to date are associated with various ecological roles in forest ecosystems. These roles include mycorrhizal associates, wood decay, plant endophytes, and plant pathogens.

DISCUSSION AND FUTURE STUDIES

LSU and ITS sequences have high utility for fungal species identification because GenBank[®] contains a large database of fungal LSU and ITS sequences. Most of the LSU and ITS sequences of root-associated fungi from *Pinus merkusii* and *P. kesiya* in the Philippines, showed a reasonably high similarity to some ITS or LSU sequences in GenBank. When both ITS and LSU regions were compared, 38 percent shared general agreement between ITS and LSU as to the closest genus-level match in GenBank[®] (Table 1). For this reason, we have reasonable confidence in the genus-level identifications for the eight isolates where both ITS and LSU provide GenBank[®] matches to the same genus. For many isolates, the ITS and LSU each matched different genera in GenBank, which indicates that DNA sequences of other regions are needed to help identify these isolates. In this preliminary study, we have validated the usefulness of DNA sequences for assessing fungal diversity in forest ecosystems. Additional studies are needed, however, to characterize species for which DNA sequence information is unavailable. The DNA sequence database of GenBank is constantly growing, and the capacity for DNA-based identification of fungal taxa continues to improve as more reference sequences become available. Fungal isolates that we could not identify to genus might not be present in GenBank[®] or they may not have been formally described yet. Only a small proportion of fungal species have been formally described to date (Hawksworth 2012). For this reason, collaborations are needed among mycologists and fungal herbaria to improve the efficacy of DNA-based identification. Our preliminary survey of root-associated fungi in the Philippines was limited to a few pine trees. Most forests in the Philippines and Pacific Islands, however, have high species diversity. Such forest biodiversity is likely associated with a large variety of forest fungi, which remain largely unexplored. We will continue to examine root-, butt-, and heart-rot fungi on hosts in diverse geographic areas of the Pacific. Once baseline data are available for species identification, additional molecular tools such as metagenomics can be used. These tools should provide improved understanding of microbial community interactions within forest ecosystems. These genetic tools may also assist in managing forests with diverse objectives and monitoring the occurrence of invasive species in the Pacific Islands.

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Table 1. Fungal isolates from the Philippines, Federated States of Micronesia, and Hawaii, USA, and their ITS and/or LSU sequence comparisons with GenBank sequences (August 2012). Blue highlighted rows indicate potential genus- or species-level agreement for ITS and LSU sequences, green highlighted rows indicate potential genus-level agreement, and white rows indicate uncertain identifications.

Isolate ID ^a	ITS1+5.8S+ITS2			LSU		
	Closest GenBank Match	GenBank Accession Number	Maximum Identity Score	Closest GenBank Match	GenBank Accession Number	Maximum Identity Score
PI-1C	Uncultured Agaricales clone WAM3	FJ179472.1	99%	<i>Mycena rubromarginata</i>	AY207245.1	98%
PI-1E	Fungal sp. ARIZ L157	FJ612709.1	95%	<i>Rattania setulifera</i> strain GUFCC15501	HM171322.1	98%
PI-2A	<i>Fusarium nematophilum</i> strain BBA70838	HQ897786.1	99%	<i>Cosmospora glabra</i>	DQ119556.1	98%
PI-2C	Fungal sp. <i>Dendrosporium</i> sp. ICMP14921	AY699695.1 EF029206.1	97% 92%	<i>Dactylaria hyalotunicata</i>	EU107298.1	94%
PI-2F	Uncultured Ascomycete clone 251.07.F05	EF619849	99%	<i>Dactylaria zapatensis</i>	EU107287.1	96%
PI-2G	Uncultured Sordariales type OTV.LH22	GQ268569.1	99%	<i>Ellisembia calyptata</i>	DQ408564.1	96%
PI-4A	<i>Penicillium inflatum</i>	AJ608959.1	99%	<i>Chaetosartorya stromatoides</i> isolate CBS 500.65	FJ358280.1	96%
PI-4C	<i>Oidiodendron maius</i>	HQ608115.1	99%	Uncultured <i>Oidiodendron</i> clone	JF519596.1	98%
PI-4D	<i>Penicillium adametzii</i>	AF034459.1	99%	<i>Chromocleista malachitea</i> isolate CBS 647.95	FJ358281.1	97%
PI-7C	<i>Diaporthe</i> sp. XL-C3	EF488448.1	99%	<i>Diaporthe eucalyptorum</i>	JX069846.1	97%
PI-7H	Uncultured Hypocreales isolate 6.2 I1	GU056003.1	99%	<i>Absidia repens</i> strain SHTH004	JN982937.1	99%
PI-9A	<i>Scytalidium</i> sp. 2013	EU334799.1	99%	<i>Crinula caliciiformis</i> isolate AFT0L-ID272	AY544680.1	95%
PI-9C	<i>Scytalidium</i> sp. 2013	EU334799.1	99%	<i>Pseudeurotium</i> sp.	AB470532.1	96%
PI-10A	<i>Diplogelasinospora inaequalis</i>	AY681201.1	99%	<i>Diplogelasinospora inaequalis</i>	AY681167.1	99%
PI-10B	<i>Trichoderma spirale</i> strain DAOM 183974	EU280068.1	100%	<i>Sphaerodes retispora</i> var. <i>retispora</i> strain CBS 994.72	GU205261.1	99%
PI-10D	<i>Pleurostomophora richardsiae</i>	AB364704.1	99%	<i>Pleurostomophora richardsiae</i> strain CBS H-7595	AY761080.1	99%
PI-10H	<i>Umbelopsis isabellina</i>	JF440625.1	99%	<i>Umbelopsis isabellina</i> strain NRRL 1757	JN940879.1	99%
PI-12B	<i>Nectria mariannaeae</i> strain JET	HM152982.1	100%	<i>Mariannaeae aquaticola</i> strain MFU090225	GQ153837.1	100%
PI-12E	<i>Neonectria radicolica</i> isolate B	HM214443.1	99%	<i>Neonectria punicea</i> strain CBS 124262	HM534901.1	95%
PI-13D	<i>Phialophora repens</i>	AF083195.1	98%	<i>Pleurostomophora repens</i> strain CBS H-7594	AY761078.1	99%
PI-17C	<i>Dendrosporium</i> sp. ICMP14921	EF029206.1	89%	<i>Hypocrea nigricans</i> strain NBRC 30611	JN941464.1	99%
PI-17E	<i>Hypocrea lixii</i> strain DAOM 234005	EU280091.1	100%	<i>Hypocrea lutea</i>	AB027384.1	97%
PI-17J	<i>Fusarium solani</i> strain ATCC 56480	FJ345352.1	99%	<i>Fusarium solani</i> strain 001AFUS	JN939570.1	100%
PI-17K	<i>Hypocrea virens</i> isolate TR039	HQ608709.1	96%	<i>Sphaerodes retispora</i> var. <i>retispora</i> strain CBS 994.72	GU205261.1	99%
PI-18G	<i>Biscogniauxia</i> sp. UFMGCB	JQ327868.1	98%	<i>Biscogniauxia nummularia</i>	GQ428318.1	93%
Hawaii-1	<i>Fuscoporia gilva</i> strain xsd08128	FJ481039.1	95%	<i>Fuscoporia gilva</i>	HQ328525.1	99%
FSM-1	<i>Fulvifomes</i> sp., Hymenochaetaceae sp.	AY558633.1	93%	NA		
FSM-2	<i>Ganoderma</i> sp.	JN234427.1	94%	NA		
FSM-3	<i>Fulvifomes</i> sp., Hymenochaetaceae sp.	AY558615.1	95%	NA		



OBSERVACIONES Y RECOMENDACIONES SOBRE LOS INSECTOS Y LAS ENFERMEDADES EN LOS BOSQUES DE URUGUAY

Will Littke¹ and Jorge Martinez Haedo-Pons²

Aforestation in Northern Uruguay began in earnest in the late 1990s with *Pinus taeda* (loblolly pine) and *Eucalyptus grandis* and *E. dunnii*. These forest plantations are now entering their first commercial thinning phase, producing veneer, saw-logs, pulp wood, and biomass for energy conversion. Early regeneration problems consisted of defoliator ants, white grubs, and *Cinara* aphids. Over a ten-year period a constant influx of both native and exotic pests became established in these forests. The complexity of forest pests is ever increasing as new habitats are created.

PINE PEST ISSUES

Several significant pests of *P. taeda* are established in Uruguay. These include *Sirex noctilio*, *Orthotomicus erosus*, *Hylurgus ligniperdi*, and *Pissodes castaneus*. Biocontrol efforts against *Sirex* has proved very effective using the nematode *Beddingia (Deladenus) siricidicola*. *Orthotomicus* is a new arrival and its biology and impacts are being studied. Observations show it is reproducing in loblolly slash and populations are currently low. It also kills single to multiple trees on stress sites. *Hylurgus* broods were found in cut stumps, but little more is known about its abundance. *Pissodes castaneus* was observed killing 1-year old loblolly seedlings in plantings near a recently thinned pine stand. Several wood decay species are prominent on loblolly slash: *Schizophyllum commune*, *Pycnoporus sanguineus*, and possibly several *Phellinus* sp. A single fruiting body of *Phaeolus schweinitzii* was found in a 30-year loblolly stand near the Argentine border.

EUCALYPTUS PEST ISSUES

Foliar disease caused by a diverse number of *Mycrosphaerella* spp., *Teratosphaeria*, and *Kirramyces* are the most common. *Puccinia psidii* (*Eucalyptus* rust) is native to Uruguay. *Ceratocystis frimbriata* is a vascular wilt pathogen observed in stressed

trees previously attacked by *Phoracantha* or ambrosia beetles. *Botryosphaeria* canker is also prevalent on pruned trees on stressed sites. Cold damage is prevalent in low topography areas. Eucalyptus plantations have seen numerous introductions of invasive pests since 2000. Currently the most serious damage is being caused by *Thaumastocoris peregrinus* (bronze bug), *Leptocybe invasa* (gall wasp), *Gonipterus scutellatus* (*Eucalyptus* weevil), *Glycaspis brimblecombei* (red gum lerp psyllid), *Phoracantha semipunctata* (*Eucalyptus* longhorn borer) and *Costalimaita ferruginea* (*Eucalyptus* leaf beetle).

CONCLUSION

Forests in Uruguay are experiencing a rapid influx of exotic and native pests as new habitats are created during forest management operations. A dedicated team of local scientists are mobilized to study impacts, life cycles and potential control methods. There is strong industry support to build a cohesive forest health network to protect this valuable forest resource.



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SHORE PINE (*PINUS CONTORTA* VAR. *CONTORTA*) DAMAGE AND MORTALITY IN SOUTHEAST ALASKA

Robin Mulvey¹, Tara Barrett², Sarah Bisbing³, and Sarah Navarro⁴

BACKGROUND

Forest Inventory Analysis (FIA) data between the measurement periods 1995-2003 and 2004-2008 revealed a significant net loss (4.6 percent) of *Pinus contorta* biomass throughout its range in SE Alaska, with greater losses for larger trees and no apparent geographic trends (Barrett and Christensen 2011). Shore pine, a distinct subspecies of lodgepole pine found on coastal and wetland sites from northern California to southeastern Alaska, was the variety of *P. contorta* present in 128 out of 130 FIA plots. In Southeast Alaska, shore pine grows on muskeg (bog/peatland) sites with high water tables, acidic soils, and unique flora and fauna. There is limited tree diversity in Southeast Alaska and few trees are able to tolerate the harsh conditions of these habitats; therefore, shore pine plays an important structural and ecological role in forested wetlands. Limited baseline information of the disease and insect agents of this non-commercial species led to this 2-year project funded by the Forest Service Forest Health Monitoring Program (2012-2013).

OBJECTIVES

1. Monitor biotic damage agents of shore pine to develop baseline knowledge & identify key damage agents.
2. Evaluate the current health status of shore pine.
3. Develop a permanent shore pine monitoring network in Southeast Alaska.

METHODS

In 2012, plot locations were randomly selected from National Wetland Inventory polygons (Palustrine

Emergent Wetland and Palustrine Scrub-Shrub) that reliably contain shore pine and were ≤ 0.5 mi of roads or trails. Satellite imagery and other GIS tools were used to assess site accessibility and forest type. 24 permanent shore pine plots were established in Juneau, Hoonah, Mitkof Is., Wrangell Is. and Prince of Wales Is. on National Forest, State, Tribal and City/Borough lands. The total plot network will contain 50 plots across these 5 locations in Southeast Alaska. The FIA plot layout was adopted to allow for comparison with the broader FIA plot network. FIA plots consist of 4 24-ft. radius subplots spaced 120 ft. apart, at 120°, 240° and 360° from the central subplot. Our plots contained 3 of 4 subplots. Prism counts, slope, aspect and percent vegetation cover were collected in each subplot.

Plot Tree Information (≥ 4.5 ft. tall; tagged for future monitoring):

All Species:

- Live/dead status
- Decay class
- DBH, ht, & live crown ht
- Crown dieback (percent)
- Wound type & severity
- Presence of decay or conks

Shore pine:

- Years of foliage retention
- Foliage disease type & severity
- Foliar-feeding insect type & severity
- Western gall rust (WGR) rating
- Incidence of WGR bole galls
- Crown dieback (percent) associated w/ WG

Foliage samples were collected for identification of foliar pathogens and insects, and are being incubated to promote fruiting body development for identification. Recent branch mortality associated with WGR was often localized within or near plots; galls were collected for identification of insects and fungi contributing to mortality of gall-infected branches.

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PRELIMINARY RESULTS ~ 2012

Across 24 plots, data was collected on 2,456 trees (≥ 4.5 ft. tall), including 510 trees ≥ 5 in in diameter. Decay class information was collected on 202 dead trees (140 dead shore pine), which may help to detect a recent pulse in mortality.

Western gall rust (WGR) was detected on 86 percent of live shore pine. Bole galls, which can lead to topkill and whole tree mortality, were observed on 33 percent of shore pine (6 percent had multiple bole infections). Topkill associated with galls was observed on 23 percent of shore pine; some had new leaders. A 1-6 scale (similar to the dwarf mistletoe rating system) was used to quantify WGR: 42 percent of shore pine were rated 1-2 (low severity), 37 percent were rated 3-4 (moderate severity), and 7 percent were rated 5-6 (high severity). Mean crown dieback associated with WGR ranged from 8 percent to 44 percent for WGR ratings of 1 and 6, respectively. WGR does not typically kill branches directly, but leads to secondary attack by insects and fungi.

Wounds (mechanical damage, frost cracks, porcupine feeding, bear scratch) were observed on 48 percent of live shore pine, and 28 percent had moderate to high severity wounds. Wound severity and the overall proportion of live trees wounded increased with

diameter class. Animal feeding and antler rub are thought to be major causes of wounding; the specific cause was often unknown.

Foliage disease or leaf mining insects were reported on 50 percent of shore pine; 31 percent had moderate or severe foliar damage. *Dothistroma pini* and lodgepole needle miner (*Coleotechnites milleri*) are common foliar damage agents and may significantly reduce needle retention. A potentially new sawfly species was observed feeding on foliage in 1/3 of plots, and larvae have been reared to adulthood for identification.

CONCLUSION

The 50-plot network will be completed in 2013, with remeasurement recurring every 4-5 years. This data will be used with FIA data to understand causes and incidence of damage and mortality in shore pine, and to help determine whether the loss of shore pine detected through FIA is part of continuing trend.

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STUMP REMOVAL FOR ROOT DISEASE CONTROL: TRIAL EXAMINATIONS IN SOUTHEASTERN BRITISH COLUMBIA

Michael Murray¹

ABSTRACT

Root disease caused by *Armillaria* spp. is a leading agent of mortality and growth loss in forest plantations of Southeastern B.C. The removal of stumps soon after tree harvesting has been promoted as a method to limit root disease in post-harvest regeneration. During the 1980s and 1990s numerous operational trials were established in Southeastern BC with stump removal treatments. Many of these trials are now being evaluated for efficacy of stump removal. Our objective is to estimate the efficacy of stump removal for ameliorating root disease. To do this, we are assessing current incidence of root disease and growth among fourteen trials. Two trials (Knappen Creek and Wetask Lake) have preliminary results. Assessments for all fourteen trials are expected to be completed in 2013.

INTRODUCTION

Stump removal has been promoted as a method to limit root disease in B.C. forests for more than 20 years, particularly for *Armillaria* root disease in the southern interior of the province. A variety of field studies to measure positive and negative effects on post-harvest regeneration has ensued. Fifty documented trials with a stump removal focus have been established in B.C. (Hannam 2012, unpublished spreadsheet). The majority of studies are in the southern interior of B.C. (37), with 9 in the coastal region and 4 in the northern region. The southern interior and adjacent northwestern U.S. have the greatest collection of stumping research sites in the world, followed by New Zealand, Great Britain and, to a much lesser extent, Sweden and Finland (Vasaitis et al. 2008).

Research sites in North America are typically divided spatially into separate treatments (stumps removed and stumps retained). The most commonly measured variables are growth (height and diameter) and forest health, especially root disease incidence. Hannam (2012) documented 16 trials in B.C. that have been measured for growth. Forest health results are formally reported for eight individual trials (Sturrock et al. 1994; Davis and Machmer 1998; Newsome et al. 2010; Leslie 2011; Cleary et al. 2013) plus a network on Vancouver Island (Zeglan 1999).

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Results from trials installed specifically for root disease often indicate significantly better growth and/or survival with removal of roots (Sturrock 2000; Vasaitis et al. 2008; Cleary et al. 2013) although results can be variable. One recent examination suggests pushover stumping does not reduce *Armillaria* root disease (Chapman et al. 2011). Another quandary - stump extraction and root raking treatments may emulate site preparation treatments that have the potential to increase growth or improve establishment and therefore confound stump removal trials; few stump removal trials have attempted to isolate this effect and data for two trials (Nine Mile Creek Trial and Big White/Greenwood Trial; Mike Curran, BCFS, personal communication, August 9, 2012) that address this issue in southeastern B.C. are just now being collected.

OBJECTIVE

Estimate the effect of stump removal treatments on:

- Incidence of root disease
- Growth of trees (diameter and height)

METHODS

In Southeastern B.C., fourteen pre-existing trials were selected for field assessment during 2009-2012 (Appendix 1). These trials were originally established (treated and planted) between 1980 As a component of each trial, there was and 1996. As a component of each trial at least one stump removal treatment and a matched stump retention area (control) for comparison. Not all trials were established exclusively to gauge root disease (e.g. Long-term Site Productivity trials), thus

trial design including site selection criteria, replication, treatments, survey methods, and measurement variables often differed.

Most trials have trees which were permanently tagged upon establishment. During 2009-2012, each trial was surveyed. Every tree was assessed for health, cause of death, diameter, and height. Data are being compiled with analysis to be in 2013. Results will be based on a meta-analysis of the fourteen sites.

PRELIMINARY RESULTS

Knappen Creek : Less Disease

Findings for the Knappen Creek trial (Figure 1) indicate a noticeable difference in incidence of *Armillaria* between the treatments (Table 1). We examined all dead trees for signs of *Armillaria* and found signs of mycelial

fans on 62.5 percent of these trees. The actual incidence was likely higher, but mycelial fan imprints disappear with natural degradation of woody tissue over time. An unexpected finding relates to the two stump removal treatments where results indicate more root disease associated with stumping followed by root raking. This is counter to the expectation of lower disease incidence wherever more inoculum sources (roots and stumps) are removed. This may be a reflection of higher incidence of root rot in the vicinity of the root-raked plots found during trial establishment (Don Norris, retired BCFS, personal communication, January 17, 2012). Differences in tree growth (height and diameter) as a reflection of treatment is not as evident – with a slightly better overall growth where stumps were removed. Further analyses are being conducted to compare responses between different tree species. Comparisons between 1997 and 2011 are also being performed.

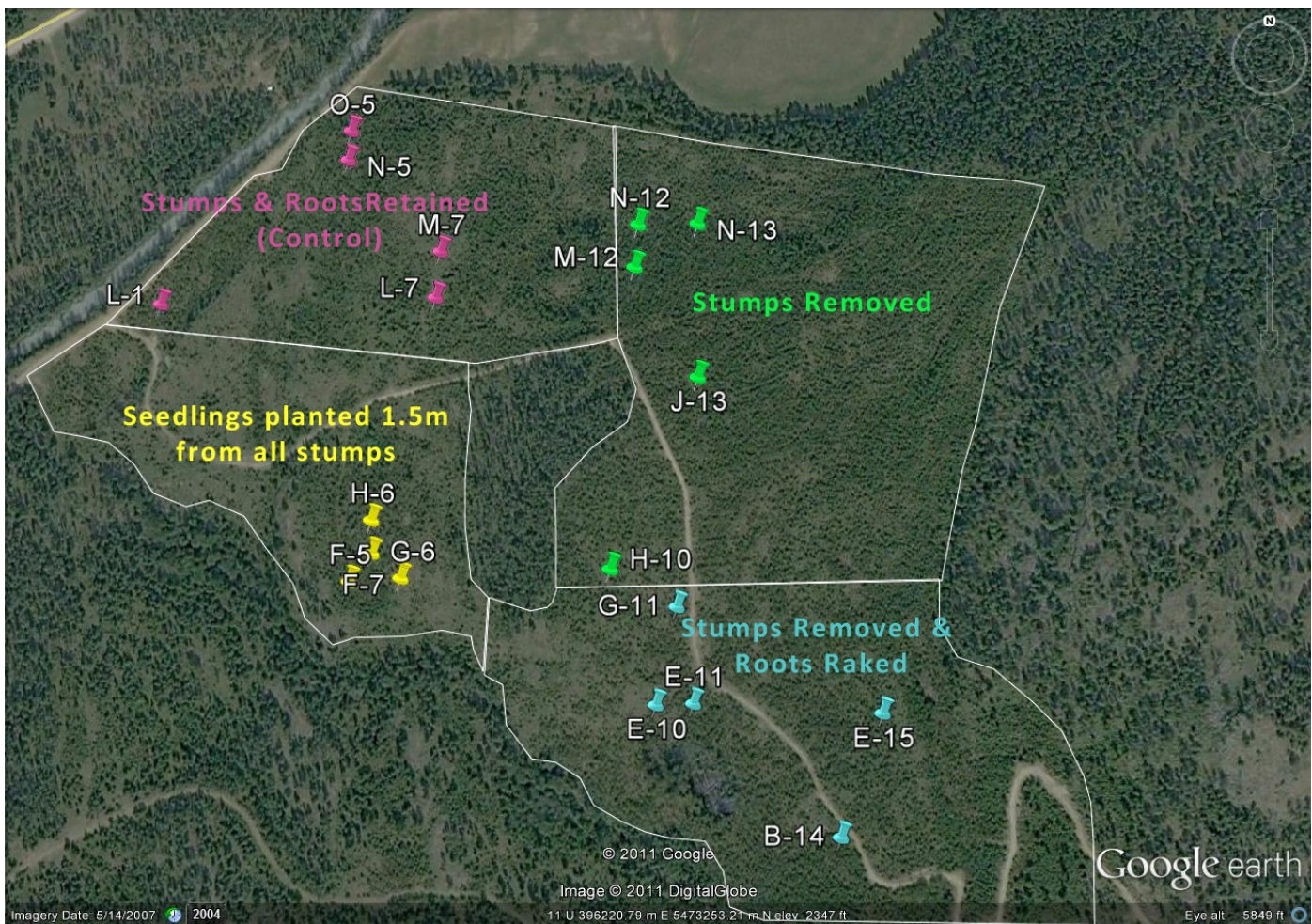


Figure 1. Knappen Creek trial plots located near Grand Forks, BC (62 miles northwest of Colville, WA).

Table 1. Knappen Creek: measured observations within each root disease treatment block.

Treatment (location in harvest unit)	Live and Dead Trees with <i>Armillaria</i> (%)	Average Growth (height / dbh)	Dead Trees (%)
Stumps removed (NE)	6.7	9.7 m / 9.1 cm	7.6
Stumps removed + roots raked (SE)	9.7	9.2 m / 9.4 cm	9.6
Stumps retained + seedlings planted 1.5m from all stumps and large roots (SW)	12.2	8.0 m / 7.8 cm	12.7
Stump & roots retained (NW)	17.0	9.3 m / 9.0 cm	16.8

Wetask Lake: Very Little *Armillaria*

Although the site was found to have a high incidence of *Armillaria* when established in 1993, the current evidence is not pronounced. In fact, neither treatment is showing much infestation, and correspondingly a difference between them is not significant (Table 2). A comparison of tree heights and diameters between control and treatment resulted in cedar, birch, and hemlock showing significantly taller and larger average DBH in the control. However, western white pine is significantly taller in the treatment area.

Table 2. Comparison of regenerating tree conditions for Wetask Lake stump removal study.

	No. Trees	Avg. ht. (cm)	Avg. DBH (cm)	No. w/ <i>Armillaria</i>
Stumps Present	1,071	475	4.18	12
Stumps Removed	1,174	421	3.83	8

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Appendix 1. Root removal trials in Southern B.C. undergoing assessment.

Trial Name	Biogeoclimatic Zone	Dominant Regeneration	Treatments	Date of Treatments	Date Trees Planted	Dates: Follow-up Assessments
Big White	Interior Douglas-fir	Lodgepole pine and Douglas- fir	1. Stumping 2. Control 3. Site preparation	1995	1996	1999, 2001, 2011
Boundary Crk.	Interior Douglas-fir	Lodgepole pine and Douglas- fir	1. Stumping 2. Control 3. Site preparation	1995	1996	1999, 2001, 2011
Columbia West	Interior Cedar-Hemlock	Douglas-fir, Spruce, Lodgepole Pine	1. Pushover 2. Stumping 3. Control	1987	1987	1998, 1999, 2011
Gates	Interior Cedar-Hemlock (ICHmk1)	Douglas-fir, W. Larch	1. Stump removal 2. Stump with root raking 3. Control	1980	1982	5yr intervals thru 2012
Knappen Crk.	Interior Cedar-Hemlock	Lodgepole Pine, W. Larch, Douglas-fir	1. Stump removal & root raking 2. Stump removal only 3. Planting 1.5m away from stump/major roots 4. Control	1990-91	1991	2011
McPhee LTSP	Interior Cedar-Hemlock	Douglas-fir, Western white pine	1. Stump removal 2. Control 3. Various LTSP treatments	2003	2004	2009, 2012
Marl Crk.	Interior Cedar-Hemlock	Lodgepole Pine, Douglas-fir	1. Stumping (bulldozer) 2. Control	1983	1985	5yr intervals thru 2012
Ninemile	Mountain Spruce (MSdk)	Lodgepole Pine, W. Larch, Spruce	1. Stumping 2. Stump (<40cm diam.) 3. Hypholoma 4. Control 5. Site Preparation (Clearcut & Shelterwood)	1997	1998	2002, 2011
Phoenix	Interior Cedar-Hemlock (ICHmk1)	Douglas-fir, W. Larch	1. Stump removal 2. Stump with root raking 3. Control	1980	1982	5yr intervals thru 2012
Rover LTSP	Interior Cedar-Hemlock	Douglas-fir, White Pine	1. Stump removal 2. Control 3. Various LTSP Soil treatments	2002	2003	2008, 2012
Sutherland Crk.	Interior Cedar-Hemlock	Lodgepole Pine, W. Larch	1. Pushover (excavator) 2. Hypholoma 3. Control (Clearcut & single tree selection)	1999	2000	2012
Wetask	Interior Cedar-Hemlock	Douglas-fir, Paper Birch, W. Larch	1. Stumps removed (excavator) 2. Control	1993	1994	1990s, 2009
Whitetail Brook	Interior Douglas-fir (incl. Mountain Spruce) (IDF dm2 & MS dk1)	Ponderosa and Lodgepole Pines, Douglas-fir, W. Larch, Spruce.	1. Pushover (excavator) 2. Hypholoma 3. Control (Group & single tree selection)	1996	Natural Regen.	2012
Zibins	Interior Cedar-Hemlock	Ponderosa Pine and W. Larch	1. Pushover (excavator) 2. Hypholoma application 3. Control (All in small clearcuts ≈1 ha)	1990s	1990s	2011



RED ALDER INOCULATIONS WITH *PHYTOPHTHORA* SPECIES FROM OREGON STREAMS: TESTING PATHOGENICITY OF THE "TREE-KILLER" GENUS

Sarah Navarro¹ and Everett Hansen¹

Described as one of the most destructive pathogens of agricultural crops and forest trees, *Phytophthora* is a genus of microorganisms containing over 100 known species. *Phytophthora alni* has caused collar and root disease in alders throughout Europe and has recently been isolated from the interior of Alaska (Adams 2008). Found throughout the coastal and Willamette Valley regions of Oregon, red alder, *Alnus rubra*, has been reported to be suffering from dieback, which prompted a survey of their overall health. With concern for the presence of *Phytophthora alni*, a survey of western Oregon riparian ecosystems (WORE) was conducted in 2010. Through various sampling methods, over 1200 individual *Phytophthora* isolates were recovered, which are representative of 22 species and 2 taxa, including *P. alni uniformis* (Sims 2012). Although these *Phytophthora* species were found in association with red alders experiencing dieback, pathogenicity tests have not been completed.

Stem inoculation trials were conducted over the course of two seasons, summer and winter, to determine the pathogenicity of 13 different species of *Phytophthora* to red alder (Table 1). Stem symptoms ranged from a slight brown discoloration to a blackening of the

stem surrounding the inoculation cut. For the summer trial, *P. siskiyouensis* produced the largest average lesion area of all *Phytophthora* species tested (371 mm²). While for the winter trial, lesions from the inoculation with *P. taxon Pgchlamydo* were the largest among the isolates used (80 mm²). Using various pathogenicity testing methods, it will be determined whether these *Phytophthora* species are the causal agents of dieback in red alder. Three additional techniques to determine pathogenicity will be evaluated using the same isolates of *Phytophthora*. Each technique involves a different inoculation method: stem, soil, fine root, and leaf inoculations. The results of these experiments will address the role of several *Phytophthora* species within the dieback of red alder in Oregon riparian areas.

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MOISTURIN AND PLANT EXTRACTS REDUCE INFECTION AND SPORULATION OF *PHYTOPHTHORA RAMORUM* ON RHODODENDRON

Ebba Peterson¹, Rick Kelsey², Dave Shaw¹, Dan Manter³, and Marion Brodhagen⁴

ABSTRACT

Phytophthora ramorum, causal agent of sudden oak death (SOD) and ramorum leaf blight, continues to cause harm to native forest communities as well as the nursery industry. Naturally produced plant compounds, hinokitiol (β -thujaplicin) and thymoquinone, isolated from the heartwood of cedars, have been shown to have zoosporicidal properties (Manter et al. 2006, 2007). In this study we sought to determine if these compounds are effective at preventing infection of rhododendron leaves by *P. ramorum* zoospores with detached leaf assays. Additionally, we pursued other potential topical treatments, including extracts of the bark from a non-bole host, California bay laurel (*Umbellularia californica*), the bark of non-hosts alder (*Alnus rubra*) and white oak (*Quercus garryana*), Engelmann spruce bark (*Picea engelmannii*), and 1 year old silver fir needles (*Abies amabilis*). Combined with a commercially available anti-transpirant, Moisturin, these compounds may provide protection against the establishment and spread of *P. ramorum*.

METHODS

Compound Preparation Procedures

Bark and Silver Fir Needle Extract Preparation. Bark samples of the four species of interest were dried and ground in a Wiley mill (40 mesh screen), then extracted with 70 percent aqueous acetone. We ground fresh, one year old silver fir needles in liquid N with a mortar and pestle before extraction with ethyl acetate. Each sample was then centrifuged, filtered, and placed under a nitrogen stream to eliminate solvent and residual moisture. The solution was filtered to remove particulates and the resultant solution dried under vacuum. All extracts and compounds were combined with 3.0 ml of 1 percent or 5 percent Moisturin diluted with DI H₂O (see Table 1) and 2.0 ml of 1 percent Tergitol-NP70 in acetone.

Bleach and Cedar Oil Compound Preparation: Solutions of hinokitiol, thymoquinone, and bleach were prepared at concentrations that failed to be phytotoxic when applied to wounded leaves (Table 1). As with the bark and needle extracts, all compounds were combined with 3.0 ml of 1 percent or 5 percent Moisturin diluted

with DI H₂O and 2.0 ml of 1 percent Tergitol-NP70 in acetone.

Control Solutions: Control solutions included one treatment with a combination of 3.0 ml 1 percent or 5 percent Moisturin and 2.0 ml 1 percent Tergitol in acetone, and one treatment in which we applied DI H₂O.

Inoculum Preparation

Cultures of *P. ramorum* (one isolate each from the NA1, NA2, and EU1 lineages) grown on 1/3X dilute V8 media were flooded with DI H₂O and gently scrapped with a rubber policeman. Sporangia were shocked into releasing zoospores by placing the spore solution at 4°C for one hour, then incubating the solution at 20°C for one to two hours. Zoospores were quantified with a hemacytometer, and diluted with DI H₂O for a final concentration of 2.5×10^4 zoospores / ml.

Inoculation Procedure and Disease Assessment

Compound Efficacy Screening and Symptom Development and Sporulation Experiments. Leaves were wounded three times on each side of the midvein. One drop of zoospore suspension was applied to each wound. In the initial screening experiment all compounds were applied to the leaf surface after wounding but before inoculation. For the symptom development and sporulation experiment leaves were painted with 10 percent Moisturin in Tergitol one day after inoculation.

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Multi-lineage and Moisturin Dose Experiments:

Leaves were left unwounded and painted with the compounds before inoculation. Six drops of inoculum were applied to the abaxial side of the leaf surface. After one minute the leaves were drained of inoculum, turn adaxial side up, then the inoculum re-applied and drained.

Nine days after inoculation the leaves were scanned and the percent lesioned area was measured in ASSESS 1.0 (American Phytopathological Society). In the symptom development and sporulation experiment, sporangial production was assessed at eight days by placing the leaves abaxial side-out in conical tubes to which DI H₂O was added. The tubes were sealed and vortexed. After vacuum filtering the solutions through five µm polycarbonate filters, we counted the number of sporangia present in 20 random microscope views at 100x.

RESULTS

Compound Efficacy Screening: The lowest amount of infection was observed with the hinokitiol and silver fir

treatments. At the concentrations tested, thymoquinone and bleach were no more protective than the Moisturin control. Of the bark extracts, white oak and alder provided the best protection, although they, too, were no more active than the Moisturin control.

Multi-lineage Experiment: The EU1 lineage consistently produced larger lesions than the NA1 and NA2 lineages for all treatments that developed lesions. In the absence of wounding no lesions occurred on the leaves treated with hinokitiol and silver fir for all 3 lineages. White oak and alder did not perform any better than the Moisturin control.

Moisturin Dose Experiment: All treatments had significantly less infection compared to the control. No concentration tested was 100 percent effective.

Symptom Development and Sporulation Experiment: There was no difference between lesion area on the control leaves and the leaves painted with Moisturin post-infection. There were significantly less sporangia produced on leaves painted with Moisturin than on the control leaves.

Table 1. Extracts or compounds used in the four different experiments designed to assess the prevention of infection by *P. ramorum* zoospores.

Compound	Compound efficacy ^a	Experiment		
		Multi-lineage ^b	Moisturin dose ^c	Symptoms and sporulation ^c
0.5g OR white oak bark (dried)	X	X		
0.5g red alder bark (dried)	X	X		
0.5g CA bay laurel bark (dried)	X			
0.5g Engelmann spruce bark (dried)	X			
5.0g silver fir needles (fresh)	X	X		
1% bleach	X			
5% hinokitiol	X	X		
0.1% thymoquinone	X			
1% Moisturin		X	X	
5% Moisturin	X		X	
10% Moisturin			X	X

^a non-control treatments were combined with 5% Moisturin; Pr-05-002 (NA2) lineage only.

^b non-control treatments were combined with 1% Moisturin; Pr-05-002 (NA2), CSL-297 (EU1), and 9650 (NA1) lineages.

^c Pr-05-002 (NA2) was the only lineage tested

CONCLUSIONS

We have identified new, alternative treatments to prevent the infection of rhododendron leaves by zoospores of the NA1, NA2, and EU1 lineages of *P. ramorum*. Moisturin may be an effective treatment when applied at high concentrations, although the lowest dose tested, 1 percent, provided significant protection from infection relative to the DI H₂O control. Still, Moisturin was not 100 percent effective at our tested concentrations. Protection was aided by the addition of plant extracts. Of those compounds tested, hinokitiol and silver fir needle extract have been shown to be the most active, completely preventing infection by zoospores in the absence of wounding.



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AN ECOLOGICAL ROLE FOR *PHYTOPHTHORA* TAXON OAKSOIL IN WESTERN OREGON RIPARIAN ECOSYSTEMS

Laura Sims¹ and Everett Hansen¹

Phytophthora taxon oaksoil, an ITS clade 6 *Phytophthora*, was collected from 58 of 88 transects in riparian alder ecosystems in western Oregon U.S. More than 500 isolates were collected from water and rhizosphere sampling between June-October 2010. Again during sampling in 2011-12 it was found that a large percentage of isolates recovered were *P.* taxon oaksoil. Stream water samples containing *P.* taxon oaksoil were collected year round with more isolates collected per liter during the summer and fall while leaves were falling and accumulating in waterways. In a field comparison study it was found that the proportion of *P.* taxon oaksoil from water was similar to the proportion of *P.* taxon oaksoil from alder leaf debris, 0.57 and 0.52 respectively. In a lab study, it was found that *P.* taxon oaksoil can sporulate and grow on dried and fresh green alder leaves and petioles floated in filtered stream water. *P.* taxon oaksoil was, repeatedly and frequently isolated from fallen alder leaves but only rarely from necrotic fine roots ; < 3 percent of the *Phytophthora* sp. recovered from sterilized root were *P.* taxon oaksoil. In addition, *P.* taxon oaksoil was not isolated from attached alder leaf material > 1 m above the surface of the water. The combined evidence suggests *P.* taxon oaksoil is growing and sporulating from alder leaf debris in riparian ecosystems in western Oregon driving up the number of propagules found in water. Little is known about the roles of *Phytophthora* species in ecosystems beyond the aggressive pathogens, but it is likely that *P.* taxon oaksoil can use plant debris such as alder leaves as a carbon source and as a substrate for asexual reproduction.

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PHYTOPHTHORA SPECIES ISOLATED FROM DISEASED ALDER TISSUE AND RIPARIAN ECOSYSTEMS IN WESTERN OREGON

Laura Sims¹ and Everett Hansen¹

A survey was conducted in western Oregon on declining riparian alder. The goals of the survey were to: (i) determine *Phytophthora* species present, (ii) monitor for *Phytophthora alni*, and (iii) assess the pathogenicity of selected *Phytophthora* species recovered from riparian ecosystems. It was important to identify the species causing disease in riparian alders. Fewer *Phytophthora* species were isolated from diseased alder tissue than from baited rhizosphere and filtered water samples. *Phytophthora* species from internal transcribed spacer (ITS) clade 7, including *P. alni* subsp. *uniformis* were more than twice as likely to be recovered from diseased woody root tissue compared to fine and fleshy roots. In general, fewer species per clade were recovered from tissue sampling than from filtered water and baited rhizosphere sampling, except ITS clade 7, with four species recovered from tissue, two from rhizosphere sampling and none from water. *Phytophthora alni* subsp. *uniformis* was only collected from diseased alder tissue. In pathogenicity tests both *P. siskiyouensis* and *P. alni* subsp. *uniformis* cankers were significantly larger than negative controls (p-value = 8.11×10^{-5} , 2.61×10^{-4} respectively). *P. taxon* Oaksoil, a species commonly recovered from water, produced cankers that did not differ from uninoculated controls (p-value = 0.619).

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HOST AND HABITAT INDEX FOR *PHYTOPHTHORA* SPECIES IN OREGON FORESTS (32 SPECIES!)

Everett Hansen¹, Paul Reeser¹, Wendy Sutton¹, and Laura Sims¹

Phytophthora species are abundant in streams in healthy forests and widespread in forest soils causing cryptic diseases, in addition to their more traditional roles as aggressive pathogens. We compiled existing Oregon records from available sources of reliably identified *Phytophthora* species from forests and forest trees and summarized the results by host and habitat (Forest Phytophthoras, <http://www.forestphytophthoras.org/>). Details of documented isolates including locations, available cultures, Genbank acquisition numbers, and citations are in the accompanying interactive database.

Thirty-two *Phytophthora* species have been identified associated with 25 host species from Oregon forests or forest trees. This total includes 19 species recovered from forest streams and 19 from forest soils, generally in the absence of noticeable disease on associated vegetation. A total of 29 *Phytophthora* species were identified from the various environments in forests. Fourteen species came from trees or forest shrubs growing in cultivated and urban environments. Only three species were unique to the latter, however, including *P. ilicis*, from cultivated holly (*Ilex*), and *P. sansomeana* and *P. taxon ceanothus* from forest nurseries. Three species, *P. gonapodyides*, *P. taxon oaksoil*, and *P. taxon salixsoil* were recovered from streams in all surveyed counties. The most widespread species causing root disease or bole cankers of trees was *P. lateralis* on Port-Orford-cedar in landscape plantings throughout the state as well as on forest trees in its limited native range. *P. cambivora* and *P. cinnamomi* were widespread but uncommon on a number of forest trees.

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GROWTH AND SURVIVAL OF SUGAR PINE THROUGH AGE 25 IN SIX PROGENY TESTS ON SITES OF LOW TO HIGH BLISTER RUST HAZARD IN SOUTHWEST OREGON

R.A. Sniezko¹, R. Danchok¹, S. Long¹, D.P. Savin¹, A. Kegley¹, J. Mayo¹, and J. Hill²

INTRODUCTION

Sugar pine (*Pinus lambertiana*) is an important, long-lived conifer native to California and Oregon forest ecosystems (Figure 1). It is one of the nine species of 5-needle pines (‘white’ pines) native to the U.S. Like the other North American white pine species, sugar pine is highly susceptible to white pine blister rust (possibly the most susceptible species), caused by the fungal pathogen *Cronartium ribicola*. Blister rust causes very high infection rates and mortality levels for sugar pine in many areas, seriously threatening the sugar pine resource in those areas. Long-term programs to develop genetic resistance to white pine blister rust in sugar pine are well underway in both California and Oregon (Kegley et al. 2004; Kinloch et al. 2012; Sniezko et al. 2004).

Studies monitoring the detailed impact of a non-native, invasive pathogen such as *C. ribicola* over time and multiple sites with known genetic composition are relatively rare. In this Forest Health Monitoring project ‘Long-term Monitoring of White Pine Blister Rust Infection and Survival at 10 Sugar Pine Evaluation Sites’ (WC-F-09-01), we examine the change in incidence of white pine blister rust infection on sugar pine trees over a 23+ year timeframe (for project overview see http://fhm.fs.fed.us/posters/posters10/monitoring_sugar_pine.pdf). Two sets of field trials using diverse genetic materials are utilized: (1) a set of progeny tests consisting of a common set of families planted on six sites.

The families are open-pollinated progeny of sugar pine parent trees previously identified as ranging from (a) highly susceptible, (b) low levels of partial resistance, or (c) major gene resistance; and (2) Provenance trails which consist of open-pollinated seedlings of

parent trees from throughout the species range; the provenance trials were established on five sites. These two sets of trials are essentially permanent plots established with known genetic composition. We focus here on the incidence of blister rust in the six progeny tests at approximately 5, 10, 15, and 25 years after planting.



Figure 1. Large sugar pines adjacent to BLM’s Boulder Test site in southwest Oregon (Photo: R.Sniezko).

MATERIALS AND METHODS

The Bureau of Land Management (BLM) (5 sites) and the Forest Service (FS) (1 site) established the six progeny test sites in southwest OR in 1982 and 1983 (Table 1). Sugar pine seedlings (‘families’) from wind-pollinated seed of 53 parent trees were planted in a randomized complete block design (four reps of 10 trees/family row plot except at FS site where non-

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contiguous row plots were used), with the families grouped into two sets (planted as adjacent trials). Thirty-six to 49 families were planted per site, with 31 families common among the six sites. Spacing was approximately 2.4m x 2.4m. The sites had been previously rated for rust hazard and encompassed sites estimated to have low, moderate and high rust hazard (Table 1). Rust hazard was based on infection levels of 50 sampled trees from natural regeneration or the number of Ribes plants per 0.01 acre within the sample area. The sites were assessed for height, survival, blister rust, and other damage at approximately 5, 10, 15 and 25 years after planting. Diameter (DBH) was recorded at the latest assessment.



Figure 2. Good growth of family ‘147’ at BLM’s Rocky test site.

RESULTS AND DISCUSSION

Growth varied among sites but was generally good across sites (Table 1). Some individual trees were more than 12 in.(30.4cm) DBH (Figure 2). Although the sites varied in their pre-planting rust hazard estimates, by age 25 all six sites had high (81.4 percent) to very high (94.8 percent) levels of blister rust infection (89.7 percent overall); rust related mortality ranged from 61.9 to 93.4 percent for the six sites (76.4 percent overall). The sites with low and medium rust hazard had lower levels of rust infection in the early years, but the difference was much less by age 15 (for some details see http://www.fs.usda.gov/Internet/FSE_

[DOCUMENTS/stelprdb5280654.pdf](http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5280654.pdf) and http://fhn.fs.fed.us/posters12/Sniezko_Long_term_Monitoring_Poster.pdf).

Approximately 60 percent of the surviving trees have stem infections, although it varied widely by site (Table 1). Mortality can lag significantly behind infection, especially for trees becoming infected at a later age when trees are larger. Further assessments will be needed in the future to determine what percent of infected trees continue to survive, as well as whether the infection levels increase further in these trials.

The high level of infection on the sites of low and moderate rust hazard was unexpected and gives rise to added concerns about the long-term viability of sugar pine populations in forest ecosystems or reforestation efforts without management (use of resistant stock, pruning of branches, etc.). Calculation of rust hazard can be a useful tool, but the results here suggest that caution be taken when extrapolating to long-term where infrequent ‘wave’ years involving more widespread dispersion of the rust under optimal conditions may lead to higher than expected infection based on the baseline local rust hazard.



Figure 3. Sugar pine with cryptic bole infection (little or no swelling), but infection confirmed via presence of aecia in cracks of the bark.

Table 1. Trial background and overview summary of sugar pine growth and survival for the subset of 31 families common to all six sites.^a

Site	Rust hazard ^d	Year sown	Year planted	Last assessment	Age 25 ^b				
					# Trees assessed ^c	# Trees alive	% Survivors w/ stem infection	Height (m)	DBH (cm)
Anchor	Moderate	1982	1983	2006	1065	366	80.3	7.61	15.2
Boulder	Moderate	1981	1982	2008	1141	407	51.6	5.68	11.3
Hayes	High	1982	1983	2010	1163	78	19.2	9.01	20.4
Jamison	Low	1981	1983	2010	1029	152 ^e	74.3	10.22	22.1
Poker	Moderate	1982	1983	2010	794	105	51.4	11.12	19.5
Overall		-	-	-	6322	1289	60.4	7.79	15.5

^a Includes trees surviving rust infection as well as other mortality.

^b Age (from planting) varies from 23 to 27 years, depending on site (Anchor and Boulder assessed earlier).

^c Excludes early post-planting mortality of 77 trees (6.2 percent) at Hayes to 393 (33.1 percent) at Poker.

^d See text for details.

^e Jamison also had high level of other mortality after age 10.

Further refinements in estimating rust hazard would be welcomed by land managers. Studies such as this one emphasize the need and high value of longer-term evaluations of ‘permanent plots’ to ground truth data for models, etc.

From personal observations, it was apparent that some bole infections were cryptic, producing little or no stem swelling or were only visible due to the presence of aecia at the time of assessment (Figure 3). Some of these cankers were at the base of the tree, but no canker was evident in the assessments through age 15. Thus field assessments probably underestimate the level of infection in sugar pine. From a management perspective, most bole cankers were low at age 15 (<1.5 m), suggesting that timely branch pruning could complement genetic resistance to increase survival. A notable number of the trees alive with cankers at the latest assessment seem to be restricting the radial growth of the canker suggesting that some form of partial resistance or tolerance may be operating (Figure 4).

Further analyses and summary of the data is ongoing. Subsequent summary of the data will examine the extent that the low level of partial resistance observed in artificial screening in the first generation provides a corresponding level in the field, as well as the durability of major gene resistance (from Cr1) that is represented in several families at these sites. Breeding to increase the level of partial resistance (and to potentially

pyramid with major gene resistance) has recently begun (for some field results see Kinloch et al. 2012).



Figure 4. Sugar pine tree at Boulder site with an old ‘vertical’ canker near the base of the tree. A number of trees alive with cankers appear to be restricting the radial growth of the cankers, allowing the trees to tolerate infection and grow.

ACKNOWLEDGMENTS

Funding from the USDA Forest Service's Forest Health Monitoring program ((WC-F-09-01), and Pacific Northwest Region's Forest Health Protection and Genetic Resource programs provided support for the project and is gratefully acknowledged. We also thank BLM (Medford District) for its recognition of the importance of these sites and its support in maintaining these valuable sites and providing personnel to assist with the assessments; as well as Forest Service employees Paula Trudeau (forest silviculturist RR-SIS NF, retired) and Brian Luis who assisted with assessments.

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***XYLELLA FASTIDIOSA* – A PARASITIC BACTERIA**

William C. Woodruff¹, Ann Brooks Gould, PhD.², and Donald Y. Kobayashi, PhD.²

ABSTRACT

Xylella fastidiosa is a parasitic bacteria that infects a variety of trees and ornamentals worldwide; causing much damage and economic loss.

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***XYLELLA FASTIDIOSA* IN BIG LEAF MAPLE (*ACER MACROPHYLLUM*) IN CA**

William C. Woodruff¹ and Donald Y. Kobayashi, PhD.²

ABSTRACT

Leaf scorch, resulting in a browning of the leaf margins, has been reported throughout much of the range of big leaf maple in California off-and-on since 1964. Recently, Rutgers University has detected the bacteria *Xylella Fastidiosa* in affected maples. Work is under way to further define the range of maple leaf scorch (MLS), isolate *X. fastidiosa* and complete Koch's Postulates.

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HETEROBASIDION RECOVERED IN SOUTHERN CALIFORNIA DRY FORESTS FROM MASTICATED STUMPS NOT TREATED WITH BORATE

Paul J. Zambino¹

INTRODUCTION

Heterobasidion (“annosus”) root disease can be a severe problem in the dry coniferous forests of southern California. Its expanding infection centers cause a legacy of ongoing mortality in stands and generate hazard trees in public recreation areas (Figure 1). Two fungal species, *Heterobasidion irregulare* and *H. occidentale*, a.k.a., “P-type and S-type *Heterobasidion annosum*”, cause the disease in its predominant hosts in southern California: pines and junipers, and true fir and Douglas-fir, respectively. Both pathogens can establish new infection centers by colonizing freshly cut stumps. A routine method for preventing new root disease infection centers has been to treat fresh stumps with borate-containing compounds. However, mastication is increasingly utilized in thinning and creates highly irregular stump surfaces that have rarely been treated with preventative fungicides. The current study tested whether such untreated stumps would become colonized by airborne *Heterobasidion* spores and thus pose a potential hazard to health and safety of future forest stands and recreation areas.

MATERIALS AND METHODS

District staff from the San Jacinto Ranger District, San Bernardino National Forest provided 26 stump sections for this study from each of two sites at Black’s Mountain (Figure 2) where conifers had been thinned by mastication in August 2009.

Sections 1-3 in. in height were cut in September 2011 to have fresh surfaces by making parallel cuts in stumps below the level of mastication. Sections were wrapped in new newsprint-type packing paper, placed in plastic bags within coolers, and transported to the San Bernardino National Forest Supervisor’s Office where they were wetted with tap water, wrapped in fresh layers of paper and held five weeks at 37°F in a tree

cooler until they could be processed. They were then incubated at 60F. Stumps were examined microscopically for presence of the distinctive *Spiniger meineckellus* asexual state after two weeks and intermittently thereafter for six weeks.



Figure 1. This wind-thrown Jeffrey pine in a southern California campground was killed by *Heterobasidion* root disease. Adjacent pines could already be infected but currently lack symptoms.

All suspected columns of colonization by *Heterobasidion* were cultured on malt extract agar containing benomyl. Cultures with typical *Heterobasidion* conidiophores were identified to species by ITS sequencing (A. Eskalen lab, UC Riverside, CA). Whenever more than one column of decay was confirmed in a stump section and multiple cultures were recovered, pieces of the cultures were paired on malt extract agar plates with themselves, with each other, and with other representatives of the same species to test for heterokaryon incompatibility. In these tests, culture pieces were separated by 2mm and 1.5 cm and areas of potential confrontation examined for fusion vs. lack of fusion.

RESULTS

Colonization of stump sections was first seen as a mycelium shimmering with conidiophores, then as the easily peeled, cream to tan mycelium pictured in Figure 3. *Heterobasidion* was easily distinguishable from

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saprophytic decay and “weed” fungi, and these latter were virtually absent from areas colonized by the pathogens.

were the fir pathogen, *H. occidentale* and one was the pine pathogen, *H. irregulare*.

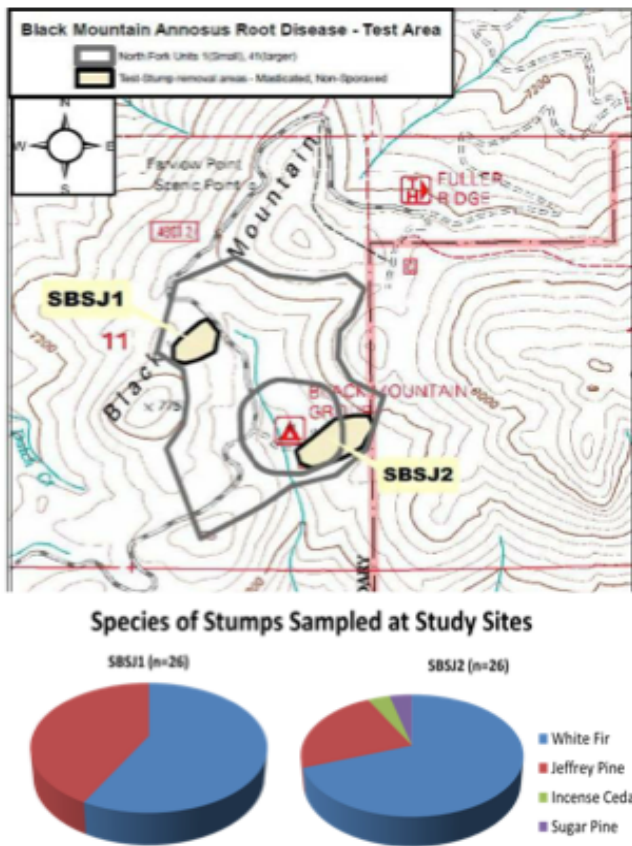


Figure 2. Stands at study sites SBSJ1 and SBSJ2 (above) on Black Mountain, San Jacinto Ranger District, San Bernardino National Forest were predominantly Jeffrey pine, white fir, and incense cedar. White fir was preferentially thinned (stumps sampled shown below).

Characteristics of *Heterobasidion* Recovery

- Recovery of the pathogens from masticated stumps was unexpectedly high: 19 percent and 42 percent of sections from sites SBSJ1 and SBSJ2, respectively.
- There was little difference in rates of stump colonization in white fir vs. Jeffrey Pine.
 - 32 percent of 34 white fir stump sections were colonized, all with the fir pathogen, *H. occidentale*.
 - 29 percent of 17 Jeffrey pine stump sections were colonized; of recovered isolates five of six

- Stump colonization was mostly from airborne spores.
 - Four stump sections had 2 strains of the same species that were incompatible with each other; multiple incompatible strains in a stump would be unlikely if colonization was through roots in an established infection center.
 - The five isolates of the fir pathogen recovered from Jeffrey pine stumps could only have entered the stumps by saprophytic growth from the surface.
 - Condition of most white fir stump sections was typical of surface colonization instead of root infection: 13 of 14 cases of *Heterobasidion* colonization in these stump sections lacked the central column of wood decay found in many firs that have had long-term heartrot from *Heterobasidion* entering through roots while trees were alive.
- The range in sizes of stumps colonized included small stumps—the sizes which can be expected to be most abundant after mastication thinning.
 - In white fir, colonized stump diameter range was 4.7-14 in., in a sampled range of 3.8-22 in..
 - In Jeffrey pine, colonized stump diameter range was 14-17 in., in a sampled range of 7-21 in.

DISCUSSION

This preliminary study indicates that masticated stumps can be easily colonized by *Heterobasidion* species via airborne spores. The potential implications of this finding will need to be determined by further studies. These should include tests of: 1) the effectiveness of different protective treatments for preventing colonization (e.g., liquid vs. powdered formulations of borate-based fungicides, vs. these same treatments on masticated stumps that have been re-cut to a smooth surface after thinning); 2) the effects of local

environment and hosts to explain site-to-site variation in colonization in southern California (e.g., measuring factors to explain differences in recovery after mastication between the two Black's Mountain sites, and comparing masticated stump colonization in mixed pine / fir stands vs. pinyon / juniper stands); and 3) the potential for *Heterobasidion* in colonized, masticated stumps to cause infection in nearby trees. Regarding this last point, even the small study reported here identified stumps that had strains pathogenic to nearest neighboring conifers, at both sites and for both hosts. For example, Jeffrey pine stump SBSJ1-20 with pine pathogen *H. irregulare* was 1 ft. from a living Jeffrey pine stem. White fir stumps SBSJ2-2 and SBSJ2-3 had fir pathogen *H. occidentale* and were 5 ft. and 7.6 ft. away from nearest-neighbor white fir stems.

Roots of these pairs of thinned and residual trees may already be in contact. But it is not known whether living roots have yet come in contact with the pathogens. Infection centers will not develop unless and until susceptible living roots contact colonized wood while

the pathogen remains viable. So, further studies with long-term monitoring of living trees next to stumps known to be colonized would be useful to understand the magnitude of the dangers of mastication, as would studies that trace columns of colonization downward from stump surfaces as they are limited by areas occupied by aggressive saprophytic competitors in root sapwood.

Despite these uncertainties, it is well known that in many situations, stumps play a major role in initiating *Heterobasidion* root disease centers. Viability of *Heterobasidion* species in stumps and roots for decades has promoted a recurrence of root disease on some sites long after stand replacement. So a prudent approach for mastication thinning in southern California will be to apply borate-based fungicides in accordance to existing rules and established practices—to treat conifer stumps as soon as they are created by management actions. It is likely that a liquid formulation able to penetrate the irregular surfaces will be a good choice for this purpose



Figure 3. Three typical stump sections colonized by *Heterobasidion* species. Left: white fir SBSJ1-23, 15 in. diameter, colonized by *H. occidentale*. Roughness at the section's edge is typical of tops of masticated stumps. Center: Jeffrey pine SBSJ1-8, 17 in. diameter, colonized by *H. occidentale*. Right: Jeffrey pine SBSJ1-20, 14 in. diameter, colonized by *H. irregulare*.



DROUGHT-MEDIATED INFLUENCE OF DWARF MISTLETOE ON LODGEPOLE PINE GROWTH

Fred Baker¹, Justin DeRose², Stefan Zeglen³, and Michelle Cleary⁴

The dwarf mistletoe *Arceuthobium americanum* is an important pathogen affecting lodgepole pine throughout its range in British Columbia (more than 12.3 mm ha). Individual infected trees have been found to be shorter and had smaller breast height diameters and total volume than uninfected trees. Reductions in height and diameter of infected lodgepole pines have also been observed, which were smaller on wet sites than on drier sites. During times of below average precipitation, both infected and uninfected tree growth decreased, but when precipitation returned to normal, uninfected tree growth recovered, while infected tree growth did not.

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ALTERNATIVES TO METHYL BROMIDE- EFFECTS OF PIC\CHLOR60 AND METAMPIC USING TIF TARP TO ACHIEVE LOW FUMIGANT BUFFER ZONES

Jerry Weiland¹, John Browning², Will Littke³, Robert Edmonds⁴, Nate Johnson⁴, and Mahsa Khorasani⁴

Forest industry and state nurseries continue to produce in excess of 500 million bare root seedlings per year for reforestation. 2009 EPA requirements for new fumigation buffer zones around bare-root fields potentially impact 15-50 percent of the growing capacity of conifer nurseries. Fumigation using methyl bromide/chloropicrin continues to be used to meet state quarantine requirements for seedling shipment. Fumigant rate reduction with virtually impermeable tarp (VIF) was successfully tested in three PNW conifer nurseries during 2008-2010. Further chemical reductions using totally impermeable tarp (TIF) is the next logical step.

METHODS

Treatments were applied fall of 2010 at Aurora and Canby nurseries situated in NW Oregon. Treatments were replicated four times at each nursery. Soils were covered with Raven brand TIF tarp. Soils were tarped for 1-month. 1+0 Douglas-fir seedlings were transplanted in randomized plots during spring 2011. Soil samples and root samples were taken periodically throughout the fumigation, transplant, and growing phase of the study. Root pathology and seedling pack yields were quantified fall 2011.

RESULTS

Soils at Aurora nursery showed elevated levels of *Fusarium* pre-fumigation and levels decreased in all fumigated plots, but not in the controls. Canby soils showed very low pre-fumigation *Fusarium* levels. *Cylindrocarpon* was present in soils at both nurseries. Root debris from the previous nursery crops (Douglas-

-fir) also contained substantial pathogen inoculum. Weed control was significantly better with fumigation. Transplant 1+0 Douglas-fir grown in fumigated soil none-the-less transport significant potential root pathogens *Fusarium* and *Cylindrocarpon* to newly fumigated fields.

MANAGEMENT IMPLICATIONS

Fall fumigation using Pic-Chlor60 or Metam-Pic with TIF tarp was effective at the rates tested for weed control, eradication of soil pathogens, and seedling production. Previous work (Leon 2009) showed the *Fusarium* complex to compose of *F. commune* and *F. oxysporum* species. Root and soil isolates of *Cylindrocarpon* were identified principally as *C. destructans* and *C. liriodendri*. In-vitro tests showed that both *Cylindrocarpon* species can colonize Douglas-fir seedling roots. Elevated levels of *Fusarium* in non-fumigated plots at Aurora resulted in mortality, while high *Cylindrocarpon* levels in non-fumigated plots at Canby significantly increased stunting and culling due to small seedlings. Results suggest these treatments can recover a large percentage of the area previously thought lost due regulatory constraints over fumigant buffer zones.

CONCLUSIONS

Methyl bromide has been the industry standard for healthy seedling production for nearly 50 years. Alternative fumigate research has been ongoing for decades, but recent improvements in fumigant placement and tarps are providing insights to potential replacement treatments. Potential exists for pathogen selection following crop culture using different soil fumigant regimes.

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TIMESYNC: INVESTIGATING TRENDS IN VEGETATION DECLINE VIA SATELLITE IMAGERY

Karen Hutten¹, Christian Torgersen², Andrea Woodard³, Warren Cohen⁴, and Justin Braaten⁵

Braaten⁵

LandTrendr and TimeSync are newly developed tools for detecting landscape change with Landsat satellite imagery. LandTrendr algorithms (Kennedy et al. 2010) detect vegetation decline and recovery from annual time series of stacked satellite imagery, and results are presented in land-change maps (Figures 1 & 2).

TimeSync software (Cohen et al. 2010) is used to observe vegetation change trajectories in imagery processed by LandTrendr. TimeSync is an interactive program that allows the user to select locations, sample sizes, and indices, and to identify trend lines in reflectance for a multi-decadal data set. Originally developed to calibrate LandTrendr algorithms by synching algorithm and human interpretations, TimeSync can now be used to graphically analyze and evaluate vegetation trends for an area of interest. Example trajectories from the Olympic Peninsula, WA, illustrate the utility of TimeSync for identifying stable forested areas (Figure 3) and forest disturbed by fire (Figure 4), wind (Figure 5), insects and disease (Figure 6). These tools provide a means for land managers and researchers to (1) monitor spatial and temporal change in forest vegetation at broad scales; (2) detect forest decline and recovery; (3) determine year of onset, duration and relative magnitude of change; (4) investigate associations with disturbance events, extreme weather or climate fluctuations, and insect and disease outbreaks; (5) identify disturbance return

cycles; and (6) fill time gaps common in ground-based surveys and standard aerial photography. TimeSync is currently being developed for public use and is accessible online (<http://timesync.forestry.oregonstate.edu/>).

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In: Browning, J. Comp. Proceedings of the 60th Annual Western International Forest Disease Work Conference; 2012 October 8-12; Tahoe City, CA. ¹School of Environmental and Forest Sciences, University of Washington, Seattle, WA. ²U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Seattle, WA. ³U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Seattle, WA. ⁴USDA Forest Service Pacific Northwest Research Station, Corvallis, OR. ⁵Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR.

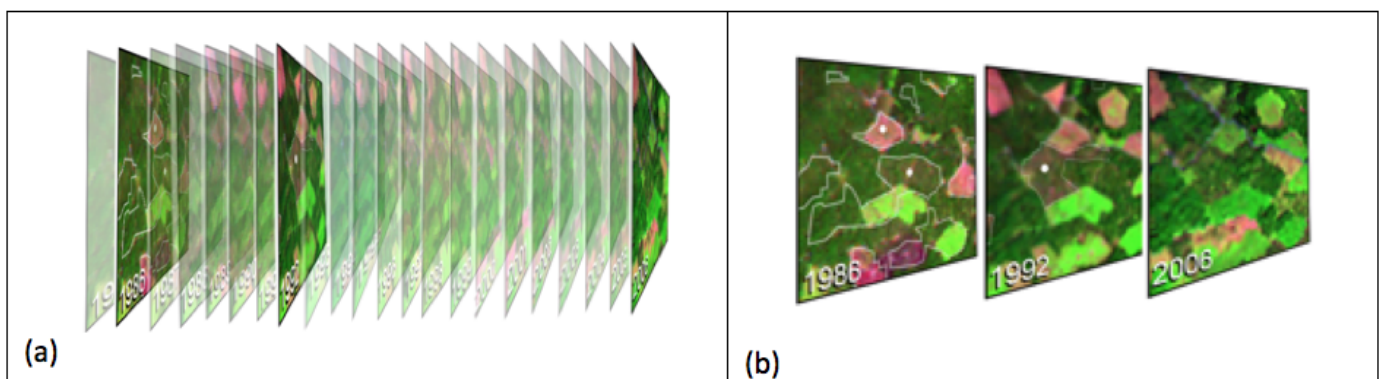


Figure 1. Graphical illustration of (left) stacked time series of satellite imagery and (right) LandTrendr change detection maps (Kennedy et al., 2010).

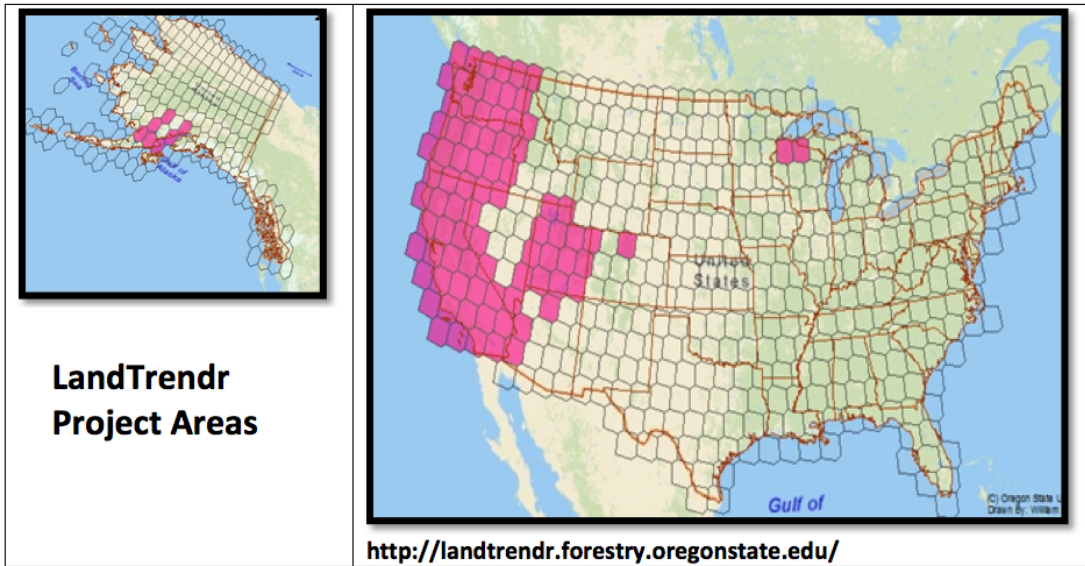


Figure 2. The LandTrendr project has processed imagery for portions of southwest Alaska and the western United States (pink shading). Temporal analysis spans the period from 1984 to 2011, and may be extended to 1972 with MSS imagery. <http://landtrendr.forestry.oregonstate.edu>.

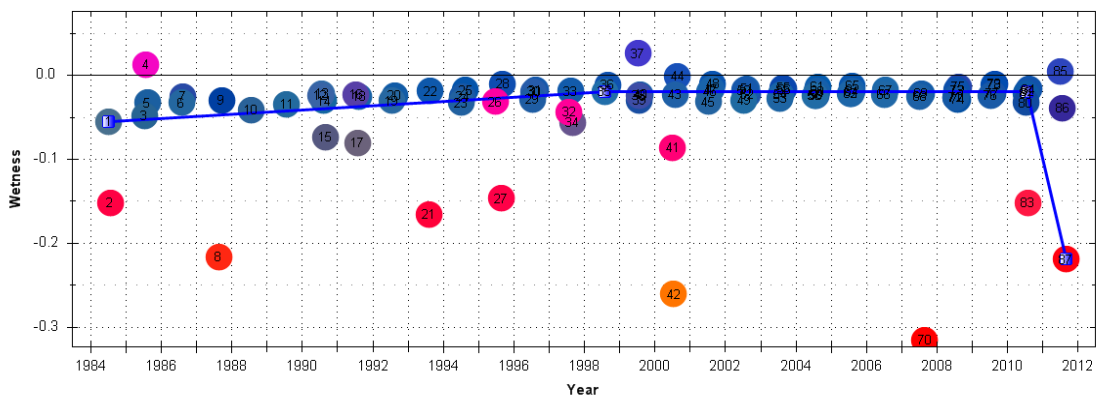


Figure 3. Example of a stable forest trajectory with growth-related incline 1984-1999, and stability 1999-2010 (false 2011 segment). Wetness on the y-axis is an index based on tasseled cap wetness. Orange, red, pink and purple circles represent images with cloud or snow cover and are not included in the trajectory.

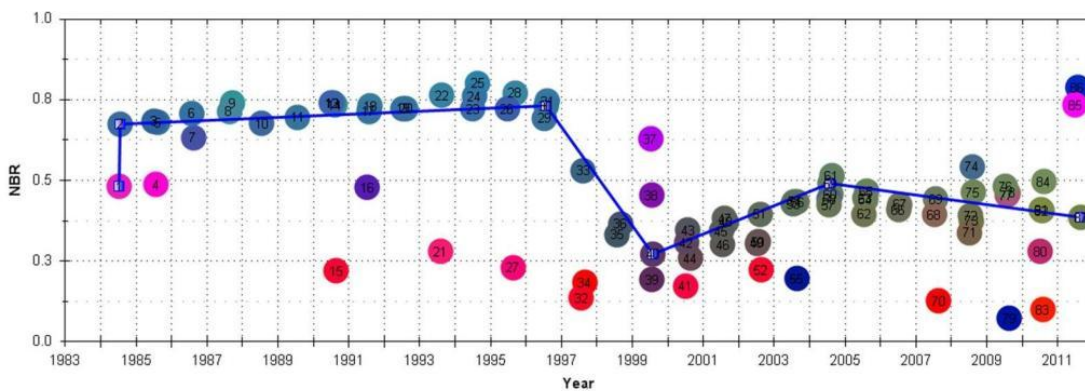


Figure 4. Example of fire effects from a 1996 fire in the Sol Duc drainage, WA, which resulted in 3 years of vegetation decline followed by recovery until 2004. Insects are likely associated with the vegetation decline after 2004. NBR on the y-axis is the Normalized Burn Ratio index. See Figure 3 for color descriptions.

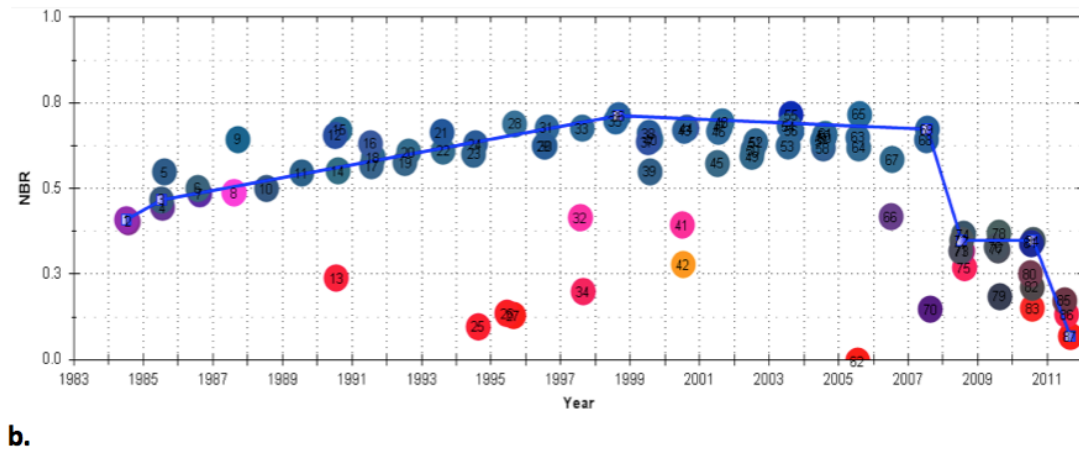
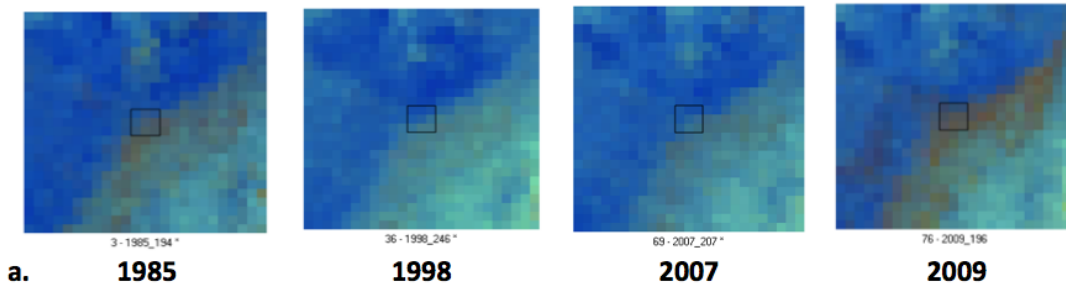


Figure 5. Example of damage from two windstorm events in the Mt. Townsend area, WA. The subset of satellite image chips (a) shows the visible color change associated with vegetation disturbance, and corresponds with the trajectory. The trajectory (b) indicates the area was damaged by wind prior to 1984, recovered and reached stability by 1999, and incurred damage from a second windstorm in 2007 (false segment 2010-2011). NBR on the y-axis is the Normalized Burn Ratio index. See Figure 3 for color descriptions.

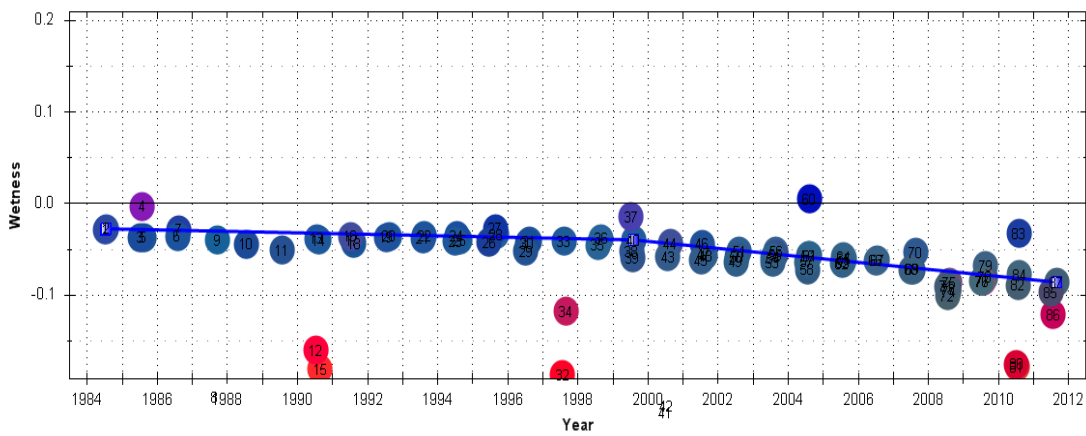


Figure 6. Insect and disease-related decline on Hurricane Ridge, WA, where balsam woolly adelgid and western balsam bark beetle are present. The decline is gradual and may have begun prior to 2000. Wetness indicated on the y-axis is an index based on tassled cap wetness. See Figure 3 for color descriptions.





PHYLOGENETIC ANALYSES OF NORTH AMERICAN POPULATIONS OF *HETEROBASIDION IRREGULARE* AND *HETEROBASIDION OCCIDENTALE**

Simon F. Shamoun¹, X. Li², J. Nie², D.L. Hammill², G. Sumampong¹, and S.H. De Boer²

ABSTRACT

Heterobasidion annosum (Fr.) Bref. *sensu lato* causes one of the most destructive diseases of conifers. *Heterobasidion* is a species complex consisting of 5 species. The North American intersterility groups (ISG), *H. irregulare* (P) and *H. occidentale* (S), were recently named. The other 3 spp., *H. annosum sensu stricto* (s.s.)- (P), *H. parviporum* (S) and *H. abietinum* (F), are European. Development of molecular diagnostics for detection & identification of *Heterobasidion* spp. in wood products for international trade is needed by the regulatory agencies (e.g., CFIA). Eight housekeeping genes were selected for genetic analysis of the 26 isolates of *Heterobasidion* from British Columbia (BC), Ontario and Quebec. Phylogenetic analyses of sequences from the 26 isolates and other 226 isolates from Europe, Asia, and North America, revealed that isolates from BC resembles *H. occidentale*, and shares sequence homology with isolates from California and Idaho. Another 16 isolates from eastern Rocky Mountains exhibited degrees of variation, and shared a close phylogenetic with *H. irregulare* isolates from Quebec, Ontario, Montana, Georgia, and Alabama. We are currently analysing the sequence data obtained from the additional housekeeping genes of the European populations of *Heterobasidion* spp. for multi-loci sequence typing.

INTRODUCTION

Root and butt rot caused by the basidiomycete *Heterobasidion annosum* (Fr.) Bref. *sensu lato* (Anamorph: *Spiniger meineckellus* (A. Olson) Stalpers) is one of the most destructive diseases of conifers in the northern boreal and temperate regions of the world. (Woodward et al. 1998). (Figures 1 and 2).

Heterobasidion is a species complex consisting of five species (Table 1). The two North American species-intersterility groups (ISG), *H. irregulare* and *H. occidentale*, were recently named and considered to be biological species (Ottosson and Garbelotto 2010). The other three species, *H. annosum sensu stricto* (s.s.), *H. parviporum* and *H. abietinum*, are European (Niemelä and Korhonen 1998). Before being given names, the different species were grouped according to their host preferences for a long time: P (pine), S (spruce) and F (fir) (Korhonen 1978; Chase and Ulrich 1988; Capreltti et al. 1990). *H. annosum s.s.* and *H. irregular* were referred to as P-ISG, *H. parviporum* and *H. occidentale*

belonged to the S-ISG and *H. abietinum* to the F-ISG. *H. annosum s.s.* is distributed almost all over Europe (Korhonen et al. 1998). *H. parviporum* occurs throughout the natural European distribution of *P. abies* and also in spruce plantations in western Europe. *H. abietinum* is found in fir forests in central and southern Europe. *H. annosum s.l.* from both S- and P- ISGs are also found in Japan and north-east Asia. In addition, several related species of *Heterobasidion* also occur in temperate conifer forests of Asia (*H. insulare*) and in tropical conifer forests of Australasia (*H. araucariae*) (Kasuga and Mitchelson 2000). The *H. insulare* complex is now considered to include *Heterobasidion linzhiense* Y.C. Dai & Korhonen (Dai et al. 2007), *Heterobasidion orientale* Tokuda, T. Hatt & Y.C Dai, *Heterobasidion ecrustosum* Tokuda, T. Hatt & Y.C Dai (Ota et al. 2006; Tokuda et al. 2009) and *Heterobasidion australe* Y.C. Dai & Korhonen (Dai and Korhonen 2009). Corner (1989) described another species, *Heterobasidion pahangense* Corner from Malaysia, which has ornamental spores, and Stalpers (1996) described the tropical American species *Heterobasidion rutilantiforme* (Murril) Stalpers.

The two North American species, *H. irregulare* (P-ISG) is found mainly in Canada (Quebec and Ontario), most of the U.S. and Mexico where pine hosts are present, Cuba, and the Dominican Republic. *H. occidentale* (S- ISG) does not occur in eastern North America, but in western North America. The known natural range is limited to western North America from

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Alaska to southern Mexico spreading east as far as the Rocky Mountains. It can colonize many tree genera, preferably *Abies*, *Pseudotsuga* and *Tsuga* (Otrosina and Garbelotto 2010).



Figure 1. Fruiting body of *Heterobasidion occidentale* (Courtesy of Dr. Simon Francis Shamoun, Canadian Forest Service, Pacific Forestry Center).



Figure 2. The pore surface of a sporophore of *Heterobasidion occidentale* (Courtesy of Dr. Simon Francis Shamoun, Canadian Forest Service, Pacific Forestry Center).

The risk of introducing a new, potentially more aggressive root and butt rot pathogen (*Heterobasidion* spp.) of coniferous trees via commercial wood products compared to the risk already present from endemic populations is not known. Development of molecular diagnostic methods for detection and identification of *Heterobasidion* species complex on import/export wood products are needed. In addition, there is an urgent demand by the pest regulatory agencies (e.g., CFIA, Canada) to evaluate the risk posed by the *Heterobasidion* species complex present in green logs and on wood products for international trade.

The specific goal of this study is use of a phylogenetic approach to examine genetic relatedness and biogeographical patterns. The focus of the current study is on the North American populations of *Heterobasidion irregulare* (P- ISG) and *Heterobasidion occidentale* (S- ISG).

The ultimate goal of this research work is to develop a miniaturized DNA amplification methodology into a hand-held device for on-site use in surveillance of *Heterobasidion* species complex and certification of wood products. The development of a hand-held diagnostic device for rapid identification of *Heterobasidion* species complex infections would be very useful both for research and effective phytosanitary recommendations for international trade purposes.

MATERIALS AND METHODS

DNA Extraction, PCR, Sequencing and Phylogenetic Data Analyses

In total, 26 isolates were collected from the coastal forests of British Columbia, and forested sites of Quebec and Ontario (Table 2). Cultures were grown on cellophane laid over agar plates. Semi-permeable cellophane (BioRad) was autoclaved and placed overtop agar plates, using 500 μ l of sterile dH₂O to adhere the cellophane to the agar plates by surface tension. *Heterobasidion* cultures were grown on malt extract agar (15 g bacto agar + 20 g ME broth/litre) with cellophane plates for ~ 2 weeks at 20°C. Mycelia were scraped and harvested from cellophane for DNA extraction (~100 mg wet weight/tube) using the Nucleospin DNA Extraction kit (Nucleospin Plant II, Macherey-Nagel, Bethlehem, PA 18020, USA; <http://www.mn-net.com/tabid/1484/default.aspx>). Eight housekeeping gene markers were selected for genetic analysis of *Heterobasidion* populations isolated in Canada. Specific gene fragments were amplified, purified, and sequenced for each of the fungal isolates. The targeted genes were transcription factor, glutathione-S-transferase, internal transcribed spacer region, NADH dehydrogenase (subunit 5), elongation factor 1 (α subunit), ATP synthase (subunit 6), glyceraldehyde 3-phosphate dehydrogenase and mitochondrial rDNA insertion element. PCR amplicons were either directly purified or gel-purified using QiaGen purification column before custom sequencing at York University using ABI automated sequencing system. ClustalW and Mega (4.0) were used to align nucleotide sequences and general phylogenetic trees.

Table 1. The *Heterobasidion annosum sensu lato* species complex*.

Species name	ISG**	Host Preference	Distribution	References
<i>H. annosum</i> (Fr.) Bref. <i>sensu stricto</i>	P	Mostly <i>Pinus sylvestris</i> , many other tree species, including <i>Juniperus</i> , <i>Picea</i> , <i>Abies</i> and several broadleaved species	Europe	Niemelä and Korhonen 1998
<i>H. parviporum</i> Niemelä & Korhonen sp. nov.	S	<i>Picea abies</i> and <i>Abies sibirica</i>	Europe and Asia	Niemelä and Korhonen 1998
<i>H. abietinum</i> Niemelä & Korhonen sp. nov.	F	<i>Abies spp.</i> , occasionally <i>Picea</i>	Southern and central Europe	Niemelä and Korhonen 1998
<i>H. irregulare</i> Otrrosina & Garbelotto nom. nov.	P	<i>Pinus spp.</i> & others	North America	Otrrosina and Garbelotto 2010
<i>H. occidentale</i> Otrrosina & Garbelotto sp. nov.	S	Many tree genera, preferably <i>Abies</i> , <i>Pseudotsuga</i> and <i>Tsuga</i>	Western North America	Otrrosina and Garbelotto 2010

*Source: 1) Niemelä, T., and Korhonen, K. 1998. Taxonomy of the Genus *Heterobasidion*. In: *Heterobasidion annosum*, Biology, Ecology, Impact and Control (Woodward, S. Stenlid, J., Karjalainen, and R., Hüttermann, A., eds.), CAB International, Wallingford, Oxon, UK, pp. 27-33. 2) Otrrosina, W.J., and Garbelotto, M. 2010. *Heterobasidion occidentale* sp. nov. and *Heterobasidion irregulare* nom. nov.: a disposition of North American *Heterobasidion* biological species. *Fungal Biology* 114: 16–25.

**ISG: Intersterility group.

Table 2. Fungal isolates, their host plants, collection sites and ISG type.

Isolate number	Collection site	Host plant	ISG type
PFC 5186	Vancouver Island, BC	Western Hemlock	S
PFC 5187	Vancouver Island, BC	Western Hemlock	S
PFC 5188	Vancouver Island, BC	Western Hemlock	S
PFC 5189	Vancouver Island, BC	Western Hemlock	S
PFC 5190	Vancouver Island, BC	Western Hemlock	S
PFC 5191	Vancouver Island, BC	Western Hemlock	S
PFC 5192	Jordan River, BC	Western Hemlock	S
PFC 5193	WFP, BC	Douglas-fir	S
PFC 5194	N/A	N/A	S
PFC 5195	Ept. Station, BC	Western Hemlock	S
PFC 5197	Ottawa, Ontario	Red Pine	P
PFC 5198	Laval, Quebec	Red Pine	P
PFC 5199	Montreal, Quebec	Red Pine	P
PFC 5200	Matawinie, Quebec	White Spruce	P
PFC 5201	Matawinie, Quebec	Red Pine	P
PFC 5202	Bois-des-Filion, Quebec	Red Pine	P
PFC 5203	Bois-des-Filion, Quebec	Red Pine	P
PFC 5204	Laval, Quebec	Red Pine	P
PFC 5205	Cedar Valley, Ontario	Red Pine	P
PFC 5206	Cedar Valley, Ontario	Red Pine	P
PFC 5207	Goodwood, Ontario	Red Pine	P
PFC 5208	Hillside, Ontario	Red Pine	P
PFC 5209	Hammond, Ontario	Balsam-fir	P
PFC 5210	Bourget, Ontario	Red Pine	P
PFC 5211	Bourget, Ontario	Eastern White Pine	P
PFC 5212	Hammond, Ontario	Red Pine	P

RESULTS AND DISCUSSIONS

Phylogenetic analyses of partial elongation factor gene sequences from the 26 Canadian isolates, along with those from 226 other strains isolated in Europe, Asia, and North America, revealed that ten isolates from British Columbia formed a cohesive cluster resembling *H. occidentale*, and shares high sequence homology with isolates from regions west of the Rocky Mountains, such as California, Oregon and Idaho in the U.S. (Appendix 1).

Another 16 isolates from regions east of the Rocky Mountains exhibited various degrees of sequence variation, and shared a close phylogenetic relationship with *H. irregulare* isolates from Quebec and Ontario in Canada, and Montana, Georgia, and Alabama in the U.S.

Phylogenetic analyses of these isolates using other housekeeping gene sequences have drawn similar conclusions (Appendix 2). These results coincided with recent studies on the taxonomy and evolutionary history of the conifer root and butt rot pathogen, *Heterobasidion* species complex (Dalman et al. 2010). We are currently analysing the sequence data in order to develop specific diagnostic tool for detection and differentiation among *Heterobasidion* species complex.

Over the past two decades, several studies have examined different aspects of the phylogenetic relationships of the different species within the *H. annosum* species complex. Kasuga et al. (1993) identified the European intersterility groups, S (*H. parviporum*), P (*H. annosum s.s.*) and F (*H. abietinum*), in the *H. annosum* species complex by ribosomal DNA fingerprinting. Harrington et al. (1998) separated the *H. annosum* species complex into three clades: the American P group (*H. irregulare*), the European P group (*H. annosum s.s.*) and the “fir” group (*H. occidentale*, *H. parviporum* and *H. abietinum*). Garbelotto et al. (1998) constructed a dendrogram of the European F (*H. abietinum*) and S (*H. parviporum*) intersterility groups using arbitrary-primed PCR and mitochondrial markers and concluded that they are both monophyletic and may lack substructuring in sub-populations. Johannesson and Stenlid (2003) focused on the European S and F intersterility groups and could identify three clades: European F, Eurasian S and North American S. The P group was studied by Linzer et al.

(2008) who showed that the Eurasian (*H. annosum s.s.*) and North American (*H. irregulare*) lineages are monophyletic sister clades. The North American P group (*H. irregulare*) was further divided into eastern and western clades; Mexican isolates showed affinity to both clades (Linzer et al. 2008). Japanese *H. annosum s.l.* isolates 18 from *Abies sachalinensis* (F. Schmidt) Mast. were found to form a subclade to *H. parviporum* (Tokuda et al. 2007).

Different factors that may be important for the speciation of the *H. annosum* species complex have been put forward, including co-evolution with the host, modern forestry, geological factors and glacial periods. Otrosina et al. (1993) proposed that *H. annosum s.l.* begun evolving its host specialization during the Tertiary period along with the more or less continuous Trans- Arctic forest that existed at that time. Linzer et al. (2008) suggest that the ancestor of *H. annosum s.s.* and *H. irregulare* was located in Eurasia and that the dispersal to North America occurred over a Beringean land bridge. They also propose that Mexico may have served as a refuge during a period of glaciation. Although several hypotheses on evolutionary scenarios for the species complex have been put forward (Otrosina et al. 1993; Linzer et al. 2008), no formal estimates of divergence times have been published previously.

The ongoing collaborative research work between the Canadian Forest Service-Pacific Forestry Center and the Canadian Food Inspection Agency, Prince Edward Island is to focus on development of molecular protocols to differentiate the species/ISG clades according to origin of the *Heterobasidion* species complex, and other interesting gene sequences of the *Heterobasidion* genome. The second objective is to develop a miniaturized DNA amplification methodology into a hand-held device for on-site use in surveillance of the pathogen in the green logs and certification of wood products according to the Canadian phytosanitary regulations.

ACKNOWLEDGMENTS

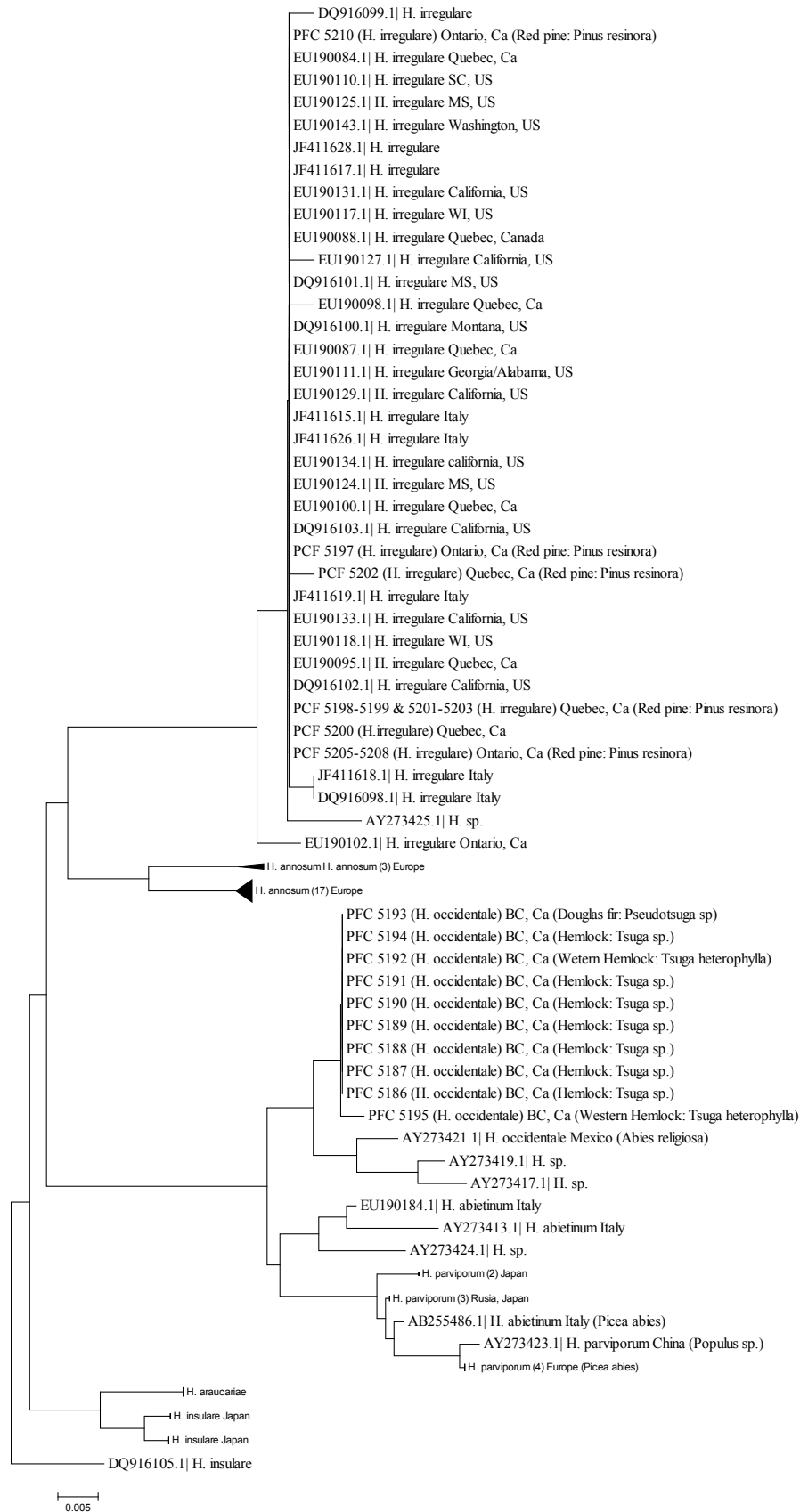
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Appendix 2. Phylogenetic representation of Canadian isolates of *Heterobasidion* and related data obtained from GeneBank based on the glyceraldehyde 3-phosphate dehydrogenase gene sequences. *H. insulare* (DQ916105) serves as the root.





COMMITTEE REPORTS



CLIMATE CHANGE COMMITTEE REPORT

Committee Chairs - Susan Frankel and Dave Shaw

The WIFDWC Climate Change Committee sponsored a panel and discussion at the 2012 WIFWC in Lake Tahoe entitled:

ASSISTED MIGRATION AND FOREST DISEASES: BOON OR LIABILITY?

An Overview of Some Potentials, Issues, and Realities of Assisted Migration for Climate Change Adaptation in Forests *Louis Iverson et al. [B-1]*

US Forest Service Policy, strategy and Practices for Assisted Migration: The State of Ambiguity and Uncertainty. *Randy Johnson et al. [B-2]*

The Role Assisted Migration Can Play in Mitigating Pathogen Threats on Forest Ecosystems Due to Climate Change *Laura Gray et al. [B-3]*

The speakers have submitted papers to this proceeding. The talks were followed by an interesting discussion regarding assisted migration implications for forest disease, and the climate change committee meeting was a continuation of this discussion, with opinions being aired. Two divergent opinions seem to pervade, one is that assisted migration is one of the only tools available to deal with climate change, while the other is that assisted migration will result in more off-site planting.



DWARF MISTLETOE COMMITTEE MEETING

Committee Chair - Fred Baker

The dwarf mistletoe committee meeting was well attended as usual. David Rusch and Leo Rankin (BC Ministry of Forests) submitted an update on lodgepole pine dwarf mistletoe after the mountain pine beetle epidemic. Many smaller diameter dwarf mistletoe infested trees survived the bark beetles, and will serve to reinfest/intensify infection in the understory. Disease incidence was 66 percent and DMR was about 1.5. These stands are young enough that dwarf mistletoe will intensify enough to affect stand growth.

Will Littke reported observations from a junket to Uruguay. He has not yet decided whether to name the *Phoradendron* after me (*bakerii*) or after the hacienda where he found it (*corona – coroneae*). . it would seem that another trip and more Coronas are needed!

After a lively discussion we adjourned to the main session. Everyone is encouraged to submit citations/links to new pubs to the mistletoe chair, for inclusion in future proceedings.



FOLIAGE AND TWIG DISEASE COMMITTEE MEETING

Committee Chair – Harry Kope (Stefan Zeglen filled in for Harry)

The committee came to order during lunch on the first day and the following presentations and discussion ensued.

PRESENTATIONS

Elytroderma Needle Blight in Montana (Blakey Lockman, USDA Forest Service, Missoula, MT)

Elytroderma needle blight (*Elytroderma deformans*) is common on ponderosa pine in western Montana and occurs in some locations in eastern Montana. Several areas of chronically severe disease exist west of the Continental Divide, causing significant decline and mortality in mature trees. Until the summer of 2012, the disease had been observed only once in lodgepole pine in Montana; a small broom was observed on a lodgepole pine growing within a stand of heavily infected ponderosa pine in northwest Montana. More recently, the disease was observed in an outlying population of lodgepole pine in north central Montana, where it is causing brooming and needle casting in several trees. This lack of infection in lodgepole pine in Montana is in great contrast to infection levels in lodgepole pine just north of Montana in Alberta and British Columbia, Canada, where the disease is common.

Several questions come to mind with this most recent observation:

- Why isn't Elytroderma more frequently infecting lodgepole pine in Montana when it is common in lodgepole pine to the north of Montana in Alberta and British Columbia?
- Is the lack of infection in lodgepole pine due to differences in populations of the tree?
- Do the populations of Elytroderma vary greatly between Montana and Canada? Could we possibly be looking at two different species of Elytroderma?
- Could this newest observation of Elytroderma in lodgepole pine in eastern Montana be an indication of a changing environment?

We plan to revisit the site in north central Montana in 2013, to determine if the disease occurs outside of the spot identified in 2012.

Swiss Needle Cast (Dave Shaw, Oregon State University, Corvallis, OR)

Swiss needle cast is a continuing problem for managers along the Oregon coast, and perhaps along the Washington coast also. Aerial detection surveys have been done since 1996 cooperatively in Oregon by the Oregon Department of Forestry and the US Forest Service, while coastal Washington was flown in 2012 for the first time since 2000 by Washington Department of Natural Resources and the US Forest Service. In 2012, 519,375 acres of Douglas-fir were observed with visible SNC symptoms in Oregon. This is an all-time high. In Washington State, 228,500 acres were observed with visible symptoms. The aerial survey is considered a qualitative survey, and should be taken as a trend.

Recent publications (pdf's) concerning Swiss Needle Cast and other information can be found at the Swiss Needle Cast Cooperative webpage: <http://sncc.forestry.oregonstate.edu/>. Some recent publications include:

Mulvey, R.L.; D.C. Shaw; and D.A. Maguire. 2013. Fertilization impacts on Swiss needle cast disease severity in Douglas-fir. *Forest Ecology and Management*. 287: 147-158.

Jayawickrama, K.J.S.; D. Shaw; and T.Z. Ye. 2012. Genetic Selection in Coastal Douglas-fir for tolerance to Swiss Needle Cast Disease. Pages 256-261 In: Snieszko, R.A. et al. (tech. coords.) Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: USDA, Forest Service, Pacific Southwest Research Station. 372 p.

Zhao, J.; D.A. Maguire; D.B. Mainwaring; and A. Kanaskie. 2012. Climatic influences on needle cohort survival mediated by Swiss needle cast in coastal Douglas-fir. *Trees*. 26:1361-1371.

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Maguire, D.A.; D.B. Mainwaring; and A. Kanaskie A. 2011. Ten-year growth and mortality in young Douglas-fir stands experiencing a range in Swiss needle cast severity. *Canadian Journal of Forest Research*. 41: 2064-2076.

Shaw, D.C.; G.M Filip; A. Kanaskie; D.A. Maguire; and W. Littke. 2011. Managing an epidemic of Swiss needle cast in the Douglas-fir region of Oregon: The Swiss Needle Cast Cooperative. *Journal of Forestry*. 109(2): 109-119.

Dothistroma Needle Blight (Alex Woods, BC Forest Service, Smithers, BC)

Alex Woods was invited to give a presentation at an international symposium on *Dothistroma* held at the University of Aberdeen in the United Kingdom. Alex gave the presentation on behalf of himself and co-author Kathy Lewis (UNBC). An abstract of the talk is provided below.

Dothistroma in Northwest BC, Canada; How far have we gone and where are we going?

Dothistroma needle blight has continued to cause extensive defoliation and mortality in plantations of lodgepole pine in northwest British Columbia, Canada. More importantly, the severity of the disease is such that mature lodgepole pine trees in the area have succumbed, and this is an unprecedented occurrence. Could climate change be responsible by surpassing an environmental threshold that has previously restricted the development of a pathogen in temperate regions? Establishing a causal relationship between climate change and local biological trends is difficult, but we have considerable knowledge of all three points of the disease triangle and we found a clear mechanistic relationship between an observed climate trend and the host: pathogen interaction. A local increase in summer precipitation appeared to be responsible and this recently observed trend might have exceeded natural fluctuations of local climate.

Further work has been conducted on the *Dothistroma* epidemic in BC, investigating in more detail the points of the disease triangle and how the host, the pathogen and/or the environment may have changed. Forest management has increased the amount of young host on the landscape in comparison to the unmanaged forest but lodgepole pine has been a natural component of these forests for millennia. A variety of mechanisms of chemical host defence and variation in genetic resistance to *Dothistroma* needle blight have been investigated but there does not appear to be a clear relationship between any single host defence mechanism and disease resistance in lodgepole pine in BC. There is evidence that in favourable environmental conditions even resistant families of lodgepole pine can be overcome by *Dothistroma*.

In terms of the pathogen and its genetic structure in BC there is evidence that sexual reproduction occurs regularly, that there is a high level of genetic diversity, and that long distance clone dispersal takes place. The implications of this work suggest that there is strong evidence for pre-existing endemic populations, a lack of evidence for new virulent genotype or for recent introduction, and high evolutionary potential.

In terms of the environment, a dendrochronological study has suggested evidence in the host tree rings of past outbreaks that date back to the early 1800s. Previous outbreaks appear to have been curtailed by unfavorable weather conditions for the pathogen. It appears that regional climate has become more favorable for *Dothistroma* resulting in more severe, widespread synchronous outbreaks. The apparent synchrony of the most recent outbreak suggests a larger climatic shift may be at play. In addition another climate variable, August minimum temperature, appears to have contributed significantly to both the current outbreak and the previous outbreaks based on dendrochronological evidence. Summer minimum temperature is known to approximate cloud cover, influencing nighttime humidity levels. In another study foliar retention, the extent of live crown and the percent of *Dothistroma* caused mortality in a 30-year-old lodgepole pine provenance trial were all related to increases in August minimum temperature. The longest daily weather record for the interior of BC, dating back to the late 1800s, indicates that both the number of summer days with precipitation and minimum summer

temperatures are on a clear statistically significant increasing trend.

The Dothistroma needle blight outbreak in NW BC continues to be monitored. The extent of defoliation and disease caused mortality has fluctuated since the time the outbreak was first observed but in general based on a series of monitoring plots the extent of healthy live foliage has declined and the cumulative percent mortality has increased. Directional climate change remains the most plausible explanation for the epidemic that has led to mature native host trees dying from a native foliar pathogen. The pathogen appears to have the capability to adapt to changing climatic conditions faster than its host. We believe the example of Dothistroma needle blight may be just one of the first clear examples of forest pathogens being affected by climate change with serious implications for future forest management.

Round Robin of Tables and Notable Notes

Ellen Goheen (USDA Forest Service, Central Point, OR) reported on two situations outside of western North America where *Phytophthora* species associated with conifer foliage are causing damage and concern. In the UK and Ireland, *Phytophthora ramorum* is causing mortality of Japanese larch (*Larix kaempferi*), a commercially important species. Millions of trees have been felled and quarantines are in place to prevent the human-assisted movement of the pathogen. *P. ramorum* also occasionally infects European larch (*Larix decidua*) and hybrid larch (*Larix x eurolepis*) in the UK (<http://www.forestry.gov.uk/forestry/INFD-8EJKP4>). In related work in Oregon, western larch (*L. occidentalis*) seedlings intentionally placed beneath *P. ramorum*-infected tanoaks are readily infected and sustain damage to both shoots and needles.

Phytophthora pluvialis, recently described from southwestern Oregon forests, has also recently been associated with needle damage and loss in *Pinus radiata* in New Zealand. No connection between these reports has been made. Bottom line: it's important to think about *Phytophthora* species when diagnosing needle damage on coniferous hosts!

Robin Mulvey (USDA Forest Service, Juneau, AK) discussed a project that she initiated in 2012 to install a

permanent plot network (50 plots) to evaluate insect and disease agents of shore pine (*Pinus contorta* var. *contorta*) across southeast Alaska. Foliage retention varied across the 25 plots installed in 2012, but was generally low due to several foliar damage agents. Foliage disease and/or leaf mining insects were detected in over half of the shore pine trees examined, and over 30 percent had foliar damage classified as moderate or severe. The most important foliar damage agents appeared to be *Dothistroma pini* and the lodgepole needle miner (*Coleotechnites milleri*). Sawfly larvae caused localized foliar damage and were observed in 1/3 of the plots; pine sawflies were previously not documented in Southeast AK and have been reared to adulthood for identification. Foliage that was collected from June to August often lacked fungal fruiting bodies to aid identification, so foliage samples have been incubated in plastic containers at 17 degrees Celsius, and incubated outside in mesh bags to promote fruiting body development. Information was requested on other methods that could be employed to promote fruiting body development for foliage disease identification.

Richard Reich (BC Forest Service, Prince George, BC) is conducting Elytroderma assessments at two progeny trials. The first generation trial is 25 years old and the second generation trial is 10 years old. Nothing riveting is happening. Richard is tracking rate of girdling and vertical spread of stem infections and timing of onset. At both sites, stem infection occurs at a very early age (~5 years of age), girdles the stems relatively rapidly, but doesn't typically cause mortality of the cambium. Richard is monitoring for impact over time, but so far there's no mortality or apparent growth loss at either progeny trial.

Richard was encouraged to plan a road trip with Blakey to try and figure out what the differences are with Elytroderma on ponderosa versus lodgepole pines and why it acts so differently in Montana.

Laura Sims (OSU) has done some work with sampling for Phytophthora that will be presented during the poster session. Willis Little (Weyerhaeuser, Federal Way, WA) mentioned that he had a poster on pest management in Uruguay that mentions foliar problems.

A report from Holly Kearns and Kristen Chadwick (USDA Forest Service, Sandy, OR) notes that in the last

10-15 years people in the Cowlitz Valley around Randal, WA have noticed significant bigleaf maple dieback and subsequent mortality. In this area, big leaf maple of all ages have been slowly declining with early leaf drop, leaf scorch, and reduced foliage size. In 2011, Washington Department of Natural Resources pathologists Dan Omdal and Amy Ramsey-Kroll

completed a survey in western Washington to look for *Verticillium* wilt as a contributing factor. Unfortunately *Verticillium* was not found to be contributing to the decline. In 2011 and 2012 samples from several trees tested positive by PCR for *Xylella fastidiosa*. They will be continuing this work in 2013.



HAZARD TREE COMMITTEE MEETING

Committee Chair- Pete Angwin

Committee Secretary- Jessie Glaeser

Approximately 40 people attended the WIFDWC Hazard Tree Committee Lunch. The meeting was chaired by Pete Angwin and Jessie Glaeser took notes. Three items were on the formal agenda:

1. 7th HAZARD TREE WORKSHOP, MAY 13-17, 2103, SEDONA, AZ.

- Mary Lou Fairweather (Local Arrangements Chair) discussed the preparations that are underway for the 7th Hazard Tree Workshop that will be held in Sedona, AZ in May, 2013. A shuttle is available from Phoenix to Sedona. The workshop will be held at a conference facility at the Relics Restaurant and Conference Center; lodging will be next door at the Sedona Real Inn and Suites. Rooms will be \$125-166 per night which includes breakfast and an open area for mixers. A block of rooms is being held until April 13, 2013. Use of the conference facility at the restaurant is free with the cost of food, including morning coffee with treat, lunch and dinner. The conference will be quite affordable.
- Planned field trips include stops in local riparian zones (Fremont cottonwoods, box elders, sycamores) and a trip to higher elevation areas towards Flagstaff. The venue is surrounded by the Coconino National Forest.
- The web site for the workshop is at <http://www.fs.fed.us/foresthealth/technology/htwc/index.htm> The first email announcement will be sent to WIFDWC members and past workshop attendees in late December.
- Program Chair is Dave Shaw. The program was discussed during a conference call of the organizing committee last April. The first day will be divided between an indoor session and afternoon field trip. The second day will consist of indoor sessions, culminating in a decay fungus identification workshop in the afternoon, led by Jessie Glaeser. A banquet, poster session and photo contest, organized by Robin Mulvey, will take place that evening. An all-day field trip will take place on the third day.

Key Topic Areas for the Indoor Sessions Include:

- Current ideas about best management practices (featuring the new International Society of Arboriculture BMP's, tips on conducting and documenting hazard tree assessments, and current equipment for decay detection. Possible invited speakers include Dr. Frank Rinn (Rinntech Inc., Germany) or Dr. Frank Telewski (Michigan State University).
- Biology and ecology of hazard tree-associated fungi (including geographical variation in root and butt rot fungal behavior, biology of riparian hardwood fungi, new knowledge concerning wood decay fungi, and *Schweinitzii* around the globe.
- Tree fall rates and characteristics after mortality of trees that die standing (when killed by Sudden Oak Death, fire, bark beetles, root rot, etc.).
- Case studies and "Top-10 Lessons Learned About Hazard Trees".

A preliminary schedule will be available soon and later finalized. Anyone who would like to help with Local Arrangements, please contact Mary Lou Fairweather.

2. ITEMS AND ISSUES FROM THE US FOREST SERVICE WASHINGTON OFFICE

Bruce Moltzan discussed the status of the FS Recreation Campground Strategic Plan, Office of General Council (OGC) advice resulting from the recent Fernandez vs. USA court decision, the status of the International Tree Failure Database (ITFD), and framework documents for hazard trees:

- The Forest Service is rethinking its strategy for placement of campgrounds. This is being looked at by OGC and being put into the Strategic Plan for Recreation.
- A lawsuit against the Forest Service (Fernandez vs. USA) was lost in September. An important legal point that was learned from the lawsuit was that if

Forest Service Manual, Handbook or other guidance documents direct that something has to be done (i.e. immediately remove a hazardous tree once it's identified), and it isn't done, or done in a timely manner, then the Forest Service can lose the "discretionary function exemption" which preserves sovereign immunity from legal action, and can become legally liable. As a result, any protocols or supplements that are being developed should be checked by OGC council, since words like "shall," "should," and "must" have critical legal meanings. Hazard tree assessments need to be documented and if written guidance documents say that trees must be removed promptly once they are marked, then this needs to be done. Regional Foresters are legally responsible for ensuring that this is done. Forest Health Protection has high visibility for training, so training materials must be inclusive and legally accurate.

John Schwandt asked if trainers should buy personal liability insurance. Bruce Moltzan was not certain of this; the Forest Service should support employees if giving training is part of their jobs. Bill Jacobi asked if it was a problem that Forest Service trains people using different standards than the ISA. Bruce Moltzan did not think this was a problem since the Forest Service offers specialty training in recreation, fire, and other areas. Dave Shaw observed that the ISA no longer uses the terms "hazard tree" or "danger tree," referring only to "risk assessment." Mary Lou Fairweather observed that fire regulations include references to hazard trees so such language cannot be changed. Robert Schlub, University of Guam, asked if Forest Service funding could be used to remove hazard trees since they are also an important problem in Guam. He was told that trees can only be removed with Forest Service funds if their removal is part of a larger treatment for insects or disease, or as part of fire suppression.

- Status of the International Tree Failure Database-The Washington Office Forest Health Protection unit continues to address ongoing problems with the database, which are primarily financial. Larry Costello (private contractor) was provided with \$10,000 to put together a needs assessment through the ISA to articulate the need for the database and how it could be used. It currently contains 6,000

accessions from FHTET; these servers will be maintained.

Strengths of the database:

- Web-based
- Cooperative with ISA
- Centralized place for data

Weaknesses of the database:

- Not enough participation or marketing
- Lack of photo database

Judy Adams has applied for \$35,000 under Special Projects to update the database. EXFOR data have also been added to it so those data are accessible. This will allow the data to be maintained for five years. The recommendation from ISA is to look for other funding sources including: ISA chapters and private industry, and U.S. Forest Service Recreation, Vegetation Management, and Cooperative Forestry staffs. A project administrator is needed who is passionate about the database and who has funding to support it. Discussion ensued:

- Committee members would like to see more training materials and applications development. Lori Winton and Robin Mulvey are applying for funds for applications development.
- John Pronos stated that the ISA needs to accept some responsibility for the database including promotion and marketing. He recommended that the ISA provide a part-time staff person to lead it while the Forest Service provides funding for five years.
- Pete Angwin mentioned that there is an ITFD steering committee and that it should have a conference call to discuss the survey results.
- Bill Jacobi asked what products can come out of the database? John Pronos answered that species profiles of hazard trees can be generated; these could possibly be put on a website. Bill Jacobi stated that at ISA regional meetings, arborists are pleading for more information and are threatening to start their own database. John Pronos mentioned that the International Database is dominated by data from California. It is currently possible to predict which tree species fail in California and which do

not. Bruce Moltzan warned that there are legal implications in identifying trees prone to failure.

- Framework Document On Hazard Tree- Bruce Moltzan described how many important plant diseases and insects, including SOD, EAB, Thousand Cankers Disease, have their own framework documents that are used to increase visibility for our priorities. He recommended the creation of such a document on Hazard Trees, noting that it is always good to have documents available to give to Congress.

3. San Dimas Hazard Tree Media Project

Pete Angwin reported on an opportunity that was raised by Jim Worrall. Funding is available to produce a handbook/training manual on Hazard Trees. Ed Messerlie hopes to assign project leader and get done in 2013. This would be of broad applicability and could be used at the national level to help train people. It would be possible to restrict content and make it more specialized so that the content applies to a general audience- for example, focusing on describing tree defects. Ed Messerlie is looking for suggestions and

feedback and wants to identify a representative from each Forest Service Region who would be willing to participate. Several members of the Committee were not initially enthusiastic. Discussion points included:

- Martin MacKenzie recommended finding out if it is marketable or needed before writing it.
- Helen Maffei observed that there are already excellent Regional hazard tree manuals available that took a lot of work to produce. In her opinion, these are much better than a general manual could be.
- Kristen Chadwick stated that it might be good to do a book about different types of tree defects, such as forks, root-sprung trees, etc., that are not covered in much detail in other manuals.

Pete Angwin concluded that it would be a good idea to have a discussion with Ed Messerlie and the designated Regional hazard tree representatives to further define what is needed and to make certain that the above concerns are not lost. A conference call will be scheduled.

Pete Angwin thanked everyone for attending and the meeting was adjourned.



NURSERY PATHOLOGY COMMITTEE MEETING

Committee Chair – Will Littke

The WIFDWC nursery pathology committee first wanted to thank Katy Mallam (USFS) for her efforts in leading the committee over the past few years. We also acknowledged her efforts along with Michelle Cram and Michelle Frank for their contributions and editorial efforts in the updated USDA Agricultural Handbook No. 680 on: Forest Nursery Pests (June 2012).

The unofficial theme for this committee meeting was the contributions of nursery pathogen identification through various PCR and DNA methodologies to nursery research. Specifically, cited were efforts on identification of isolates of *Pythium*, *Fusarium*, and *Cylindrocarpon*. Efforts have been underway supported by research funding from USDA ARS Area Wide Methyl Bromide Alternatives since 2007 to test new combinations of fumigants, however breakthroughs in DNA identification were necessary to quantify specific pathogen complexes being controlled.

Anna Leon (PhD candidate WSU, advisor Gary Chastagner) presented her research on *Fusarium commune* and the potential for PCR based methodology to quantify soil populations in Douglas-fir nurseries. This method could potentially replace time consuming and less accurate soil assay plate counts using *Fusarium* specific medias (i.e. Komada's Media). This methodology also shows promise in quantifying root infections by this pathogen. Previous work on pre- and post fumigation soil and root assays showed that current assay methods isolate a wide variety of *Fusarium* species, many with unknown pathogenicity towards conifers.

Mhasa Korsani (Ms candidate UW, advisor Bob Edmonds) illustrated her progress in identification of various *Cylindrocarpon* isolates found in Pacific Northwest conifer bare-root nurseries. Also present were Ned Klopfenstein, John Hanna, and Amy Ross-Davis (USFS Moscow) who performed some of the first PCR identifications of *Cylindrocarpon* isolates from Douglas-fir. Mhasa presented some overview of the abundance of *C. destructans* and *C. liriodendri* amounts isolates from WA and OR nurseries. *C. destructans* is well known in the literature as a regeneration pathogen,

but little is known about other *Cylindrocarpon* species. She will further explore physiological traits of isolates and tolerance to fungicides as part of her graduate work.

Highlights of the 2010 ARS study to identify alternatives to MB were presented by Will Littke and John Browning. Low rate fumigants (25-foot buffer rates) using TIF (totally impermeable tarp) composed of combinations of Chloropicrin with metam-sodium or Telone were tested in Oregon at two nurseries. Fumigation effects were comparable to MBC 250 lbs/ac under TIF for stock yield, morphology, disease and weed control. Non-fumigated seedlings were significantly smaller with higher disease development. At one nursery, control seedlings were consistently infected with *Fusarium* while another nursery had high levels of *Cylindrocarpon*. Transplant seedlings used in the trial have been implicated in reinoculation of fumigated soil with disease pathogens. However, transplant seedlings are all raised from seed in freshly fumigated soil. Four biocontrol agents (*Bacillus*, *Trichoderma*, *Streptomyces*, and *Gliocladium*) were not effective at reducing root infection when drenched three times during the growing season. Naturally occurring root fluorescent *Pseudomonad* bacteria were implicated in "blocking" applied biocontrol agents from colonizing Douglas-fir roots, but not root pathogenic fungi. a poster was presented on the results.

REFERENCES

- Stewart, J.E.; Abdo, Z.; Dumroese, R.K. and others. 2012. Virulence of *Fusarium oxysporum* and *F. commune* to Douglas-fir (*Pseudotsuga menziesii*) seedlings. *Forest Pathology*. 42:220-228.
- Kim, M.-S.; Stewart, J.E.; Dumroese, R.K.; Klopfenstein, N.B. 2012. Occurrence of the root rot pathogen, *Fusarium commune*, in forest nurseries of the midwestern and western United States. *Journal of Phytopathology*. 160:112-114.
- Dumroese, R.K.; Kim, M.-S.; James, R.L. 2012. *Fusarium oxysporum* Protects Douglas-fir (*Pseudotsuga menziesii*) Seedlings from Root Disease Caused by *Fusarium commune*. *Plant Pathology Journal*. 28:311-316.

ROOT DISEASE COMMITTEE REPORT

Committee Chair- Blakey Lockman

The Root Disease Committee met for breakfast on Friday, October 12. Thirty people were in attendance.

AGENDA ITEMS

Blakey gave thanks to Michelle Cleary for her time as chair. Michelle is now permanently residing in Sweden and will not be able to attend WIFDWC on a regular basis. She asked Blakey to take over as chair- Blakey is excited to do so and greatly appreciates the opportunity to chair this committee. She asks members to let her know of any topics they would like to discuss at future meetings.

Topics currently planned for 2013 include:

- Discussion on nomenclature of *Phellinus weirii*, *Phellinus sulphurascens*, *Inonotus sulphurascens*-led by Rona Sturrock and Everett Hansen.

Copies of 2011 minutes Root Disease Committee Report and copies of *Armillaria* TAXON article provided at the meeting.

PRESENTATIONS

1. Status of *A. ostoyae* vs. *A. solidipes* (Blakey Lockman presenting R. Hunt and D. Morrison summary):
 - Proposal to conserve “*ostoyae*” published in TAXON in 2011, rendering *A. ostoyae* the only legal name to use.
 - (S.A. Redhead, J. Berube, M.R. Cleary, O. Holdenrieder, R.S. Hunt, K. Korhonen, H. Marxmuller, and D.J. Morrison. 2011. (2033) Proposal to conserve *Armillariella ostoyae* (*Armillaria ostoyae*) against *Agaricus obscurus*, *Agaricus occultans*, and *Armillaria solidipes* (Basidiomycota). *Taxon* 60 (6): 1770-1771).
 - Decision will occur at the next International Code of Botanical Nomenclature meeting in 2016- with a prior review by a fungal subcommittee.
 - Taxon code article trumps all others until a decision is made at the 2016 ICBN meeting.
 - At this point, *A. solidipes* is a dead name!

More updates on Armillaria nomenclature!

- NABS X (North American biological species X), now a new species: *Armillaria altimontana*
- Nicholas J. Brazee, Beatriz Ortiz-Santana, Mark T. Banil, and Daniel L. Lindner. 2012. *Armillaria altimontana*, a new species from the western interior of North America. *Mycologia*, 104(5), 2012, pp. 1200–1205.

2. Update on *Phytophthora lateralis* resistance work in Port Orford Cedar (Richard Sniezko).

Richard provided an informal update, including a list of recent summary papers of seedling screening and field tests. These papers are in the proceedings of a Resistance Workshop. They provide a good summary of the progress to date, questions to answer, etc.

Sniezko, Richard A.; Yanchuk, Alvin D.; Kliejunas, John T.; Palmieri, Katharine M.; Alexander, Janice M.; Frankel, Susan J., tech. coords. 2012. Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture. p.372.

Specific papers of interest from these proceedings include:

Operational Program to Develop *Phytophthora lateralis*-Resistant Populations of Port-Orford-cedar (*Chamaecyparis lawsoniana*). http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_065.pdf. R.A. Sniezko, J. Hamlin, and E.M. Hansen.

Methods for Screening Port-Orford-Cedar for Resistance to *Phytophthora lateralis*. http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_181.pdf. Everett M. Hansen, Paul Reeser, Wendy Sutton, and Richard A. Sniezko.

Nine Year Survival of 16 *Phytophthora lateralis* Resistant and Susceptible Port-Orford-Cedar Families in a Southern Oregon Field Trial. http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_348.pdf. *Richard A. Snieszko, Jim Hamlin, Everett Hansen, and Sunny Lucas.*

Operational Disease Screening Program for Resistance to Wilt in *Acacia koa* in Hawaii. http://www.fs.fed.us/psw/publications/documents/psw_gtr240/psw_gtr240_065.pdf. *Nick Dudley, Robert James, Richard Snieszko, Phil Cannon, Aileen Yeh, Tyler Jones, and Michael Kaufmann.*

Additional discussion followed Richard's presentation:

- USFS Region 5- much POC seed was used for regenerating fire impacted lands. Also, R5 has a new GIS layer of POC.
- Oregon Department of Forestry- their seedbank is meeting the demand on non-federal lands
- There is a serious lack of natural regeneration.

ANNOUNCEMENTS

National SAF Meeting being held in Spokane on October 24-27, 2012.

- Bruce Moltzan selected as chair of the Insect and Disease Committee (D5 Committee).

IUFRO- *Phytophthora* meeting this past September- Ellen Goheen and Susan Frankel attended.

APS- meeting with MSA (Mycological Society of America) next year (2013).

- August 2012 meeting was in Rhode Island
- Very few pathologists usually attend- Jessie Micales-Glaser encouraging WIFDWC members to participate.
- August 2013 in Austin, TX (August 10-14)

The 10th International Congress of Plant Pathology (ICPP) - August 25-30, 2013 in Beijing, China- it meets every 5 years.

REGIONAL REPORTS

British Columbia

- Briefly summarizing the situation in BC:
- With the feds, Mike Cruickshank and Rona Sturrock continue to work on *Armillaria* and *Phellinus*, respectively. Both are finding it difficult to continue their work due to shifting priorities by NRCanada. Rona and Michelle Cleary are analyzing data from their project in the southern interior of BC looking at the growth impacts of *Phellinus* and *Armillaria* in mature stands across several ecological types.
- With the province,
 - o Stefan Zeglen has a five year trial comparing post-stump removal regeneration growth on treated and untreated sites.
 - o Alex Woods and Richard Reich have several *I. tomentosus* trials in the northern part of the province that are 15-20 years old and are showing results for those with patience.
 - o Michael Murray has 14 trials (8 to 20 years old), many installed by Don Norris, looking at stumping treatments for *Armillaria* that he is spending time reassessing. He has a poster with some results at this year's meeting. Also testing tree-ring techniques to: 1) age infections and 2) explore relationships with tree stress and climate.
 - o David Rusch has joined the pathology team this year and will be working primarily with *Armillaria* in the central portion of the province.

Mexico (Phil Cannon reporting)

- *P. cinnamomi* a worry on oaks
- *Armillaria* in peach orchards (unknown species)
- *Heterobasidium* sp. a known pathogen
- *Armillaria gallica* an aggressive pathogen
- Time is being spent looking at *Armillaria spp.* on avocado, including the taxonomics

USFS- WO- Bruce Moltzan

Briefly discussed the National Root Disease Paper- Bruce is willing to help Holly Kearns and Blakey Lockman with an editor he has access to. Blakey and Holly assured Bruce they were still planning to complete the paper, but have been pulled onto other

projects. The hope is to get back to it and finish it up this calendar year.

- Eric Smith- he's working on distribution maps of insects and diseases, so we should be able to tap into his effort for the Root Disease Paper.
- Discussion about PER (Pest Event Reporter) and the necessity of inputting into this database. We also discussed some of the problems with PER. It's important to document each year. Some discussion on mortality vs growth- PER is focused on mortality with is only one piece of the story with root diseases.

USFS- Northern Region

- John Schwandt- Sue Hagle now has emeritus status.
- Blakey Lockman- the future of over 1200 permanent Forest Health plots initially established by Sue Hagle is being discussed in the Region. We're trying to find a way to get the data from these plots analyzed and written up. Once that is completed, then the future of the plots can be discussed. No decision has been made on whether to continue maintaining any of these plots. Blakey continuing to work with Holly Kearns in finishing up the National Root Disease Paper. They have been editing and formatting contributions from numerous FHP forest pathologists from across the US.
- Paul Zambino- reporting that *Heterobasidion* (both *irregulare* and *occidentale*) are being detected in masticated stumps (Where? How?)
- Marcus Jackson- Working on various aspects of *I. tomentosus*. We are starting to find it more and more east of the Continental Divide, specifically in campgrounds. We hope to focus some time working on this root disease over the next few years.

USFS-Rocky Mountain Research Station, Moscow, ID. Ned Klopfenstein and Amy Ross-Davis

A collaborative STDP project between RMRS Moscow, Idaho and Regions 6 (Helen Maffei) and 3 (Mary Lou Fairweather) to develop predictive models for *Armillaria* species in the inland western U.S.A. is ongoing. Surveys in Oregon have been completed and surveys in Idaho and Arizona are nearly complete. We hope to incorporate surveys from Montana, Wyoming, and Colorado (Regions 1 and 2) into the predictive models.

Preliminary findings from Rubén Damian Elias Roman (graduate student from Colegio de Postgraduados, Montecillo, Mexico), who performed DNA-based diagnostics in our laboratory for his Ph.D. research, indicate that damage in peach orchards in Mexico is caused primarily by an undescribed species of *Armillaria*. *A. mellea* and *A. gallica* were also found on diseased peach trees, but much less frequently than the undescribed species.

Other studies are ongoing to examine the transcriptome (expressed genome determined from mRNA) from a mycelial fan of *Armillaria solidipes* (= *A. ostoyae*) on grand fir. A goal of this project is to identify genes associated with pathogenicity and adaptation. A draft manuscript has been prepared with planned submission to *Fungal Genomes and Biology*.

International collaborations are continuing on a long-term project to examine the evolutionary relationships among *A. solidipes* and related species from world-wide sources. Preliminary findings suggest that currently recognized species of *Armillaria* may actually represent a series of species complexes.

PUBLICATIONS

Ross-Davis, A.L., Hanna, J.W., Kim, M.-S., Klopfenstein, N.B. 2012. Advances toward DNA-based identification and phylogeny of North American *Armillaria* species using elongation factor-1 alpha gene. *Mycoscience* 53: 161-165.

Klopfenstein, N.B., Kim, M.-S., Hanna, J.W., Ross-Davis, A.L., Ashiglar, S.M., McDonald, G.I. 2012. *Armillaria* species: Primary drivers of forest ecosystem processes and potential impacts of climate change. pp. 53-76 in *Proceedings of the 2012 International Conference on Etiology, Ecology and Integrated Management of Forest and Fruit Tree Diseases*. 24-25 May 2012, National Taiwan University, Taipei, Taiwan.

WWETAC (Western Wildland Environmental Threat Assessment Center- Prineville, OR. Nancy Grulke, Director)

- Working on invasives- both native and exotic
- Root and butt rot surveys in the South Pacific with Phil Cannon
- Working on imagery using Helen Maffei's plots

- Vegetation modeling- incorporating insects and diseases into a dynamic model- bark beetles and root diseases the next step, especially relative to carbon.
- Request for Proposals (RFPs) due in the spring, but can send proposals to Nancy anytime

Oregon State University

- Dave Shaw-Phytophthoras are his main focus these days. Looking at old plot systems (Walt Thies, etc)- trying to determine the fate of the plots. USFS Region 6 pathologists planning to take on the maintenance of these plots
- University of Washington- Bob Edmonds. Focusing on laminated root disease. Research has come to a halt

USFS- Pacific Northwest Region

- Greg Filip- Working on writing up long term projects- 30 year Armillaria project, hope to publish results, 20 year Armillaria project- Lake of the Woods.
- Helen Maffei- Working on mapping areas of know existence of root disease- it's a real challenge mapping known and unknown areas

USFS- Pacific Southwest Region

- Pete Angwin- Klamath- BS permanent plots- Douglas-fir- up for remeasurement- submitted an FHM EM proposal to remeasure and to establish new plots
- Bill Woodruff- Bill Otrrosina is talking about retiring. Working with Matteo Garbelotto in calibrating DNA work

Colorado

- Bill Jacobi (Colorado State University)
- Jim Blodgett (USFS FHP Rapid City, SD) working on Armillaria distribution in Wyoming. Plans for expanding subalpine fir decline plots into northern Colorado

Weyerhaeuser

- Will Littke- *P. weirii*/alder rotation plots- 35 years of data collected, but haven't seen the data or its analysis

Thank you to everyone for your participation and for continuing your very important root disease work!

Notes transcribed and respectfully submitted by Blakey Lockman with additional editing from meeting participants.



RUST COMMITTEE REPORT

Committee Chair – Helen Maffei

The rust committee meeting started off with 3 formal presentations. There were approximately 30 attendees.

1. Brian Geils (RMRS, Flagstaff, AZ) shared his observations and perspectives on rust research in the west over his long career (he retired in 2012).
2. Robin Mulvey (FHP, Juneau, AK) gave an exciting power point presentation (available upon request) on Spruce Needle Rust (*Chrysomyxa ledicola*) and secondary invaders of gall rust. Her images were very dramatic (from the Kachemak Bay, Soldotna, Lake Clark NP and elsewhere); the needle rust spores almost looked like orange paint floating in the water.
3. Helen Maffei (FHP, Bend, OR) provided an overview of the process used in 2013 national insect and disease risk map (NIDRM) to model the hazard posed by important rust pathogens (available upon request). Because of the finer scale of host vegetation layer it was possible to map the risk at a finer scale as well.

UPDATES FROM ATTENDEES

John Schwant (also retired in 2012). Described several projects related to whitebark pine (*Pinus albicaulis*) (WBP) and western white pine (*Pinus monticola*) (WWP), as well as the WBP restoration program and where it is going (or not).

Michael Murray. Described British Columbia's new effort to rust screen WBP.

Betsy Goodrich. Northern AZ University has been doing with FHP Region 3 on southwestern white pine gene conservation and some other research, including some more EM plots put in this year

Dorena Tree Improvement Center Report (Richard Sniezko). Screening for genetic resistance to white pine blister rust continues at Dorena Genetic Resource Center – western white pine, sugar pine (SP) and whitebark pine are the main focus, but limber pine, southwestern white pine, and the two bristlecone pine species are also being examined (in conjunction with R3, RMRS, R2). In addition, data from field trials with WWP and SP is being collected and summarized (see some results in papers listed below). Several new restoration plantings of whitebark pine were established in 2012 on National Park and National Forest lands, and a genetic study was superimposed over the plantings. Several new WWP field trials (using the latest resistant seedlots) are scheduled with cooperators in WA and British Columbia. Control crosses continue in WWP, and the resistance screening results from the first of these advanced generation crosses is encouraging. WWP seed from BLM's Horning seed orchard is now under test.

The proceedings from the 'Disease and insect resistance in forest trees' workshop was printed in late 2012. Below are the papers dealing with white pine blister rust.

Sniezko, Richard A.; Yanchuk, Alvin D.; Kliejunas, John T.; Palmieri, Katharine M.; Alexander, Janice M.; Frankel, Susan J., tech. coords. 2012. Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture. 372 p. http://www.fs.fed.us/psw/publications/documents/psw_gtr240/.

STUDENT AWARDS COMMITTEE REPORT

Committee - Bill Jacobi, Holly Kearns, Blakey Lockman, and John Schwandt

With the proceeds from last year's Silent Auction in Leavenworth, WA we provided \$1,600 for a total of four Student Travel Awards. The 2012 Student Travel Award recipients were Betsy Goodrich (\$400, Northern Arizona University), Mahsa Khorasani (\$400, University of Washington), Sarah Navarro (\$400, Oregon State University), and Cassandra Swett (\$400, University of California Davis).

Our third annual Silent Auction was a big success raising \$1,190 through the sale of 68 individually donated items plus donations of books and cash. We want to sincerely thank the many people who brought historic documents and books, homemade food items, and handmade cloth items, jewelry, etc. to be auctioned.

Thank you to everyone who participated so generously in the auction. The balance in the Student Award Fund is now \$1,629, which will provide assistance to several students for the 2013 meeting in Waterton Lakes National Park, Alberta.

Robin Mulvey and Alex Woods joined the Student Awards Committee replacing original members, Blakey Lockman and John Schwandt.



WIFDWC PRE-MEETING FIELD REPORT: TRIP TO BARKER PASS ROAD AND GRANLIBAKKEN, LAKE TAHOE, CA

Will Littke

Teams of skilled forest health professionals descended upon the forests of Lake Tahoe region to test their skill and cunning at collecting examples of forest diseases and other specimens (Photo 1). Armed with only a dull hatchet, pocket knives and hand-lenses this group of enthusiastic collectors ventured into a wasteland of dead and dying red fir, ponderosa pine, lodgepole pine, white pine and assorted hardwoods. But first- A “sermon from the stump” on what and where to collect, how to

annotate signs and symptoms, and what time collections were due back at the meeting room (photo 2). Nonetheless, there was the typical congeniality associated with the WIFWDC community collecting specimens. Samples were labeled and displayed before being judged by a distinguished international panel of forest pathology experts. A partial list was compiled from the specimens collected (Table 1). Fun was had by all!



Photo 1. Barker Pass road area was a mixture of conifer and hardwood species as the setting for the pre-WIFWDC field trip. **Photo 2.** Phil Cannon “sermon from the stump” speech on objectives and rules for specimen collection.



Photo 3. Introduction to white pine blister rust study Barker Pass road site (Left). Just a “dead” tree photo because they’re so beautiful! (Right).

Table 1. Partial list of the specimens collected during the 2012 WIFDWC pre-meeting field Trip to Barker Pass Road and Granlibakken. (See Photos 4-6).

	Species	Family	Guild	Host
1	<i>Arceuthobium abietinum</i>	Santalaceae	parasitic plant	Red Fir
2	<i>Arceuthobium americanum</i>	Santalaceae	parasitic plant	Lodgepole
3	<i>Arceuthobium viginatum</i>	Santalaceae	parasitic plant	Ponderosa
4	<i>Cantharellus sp.</i>	Cantharellaceae	mycorrhizal fungus	conifers
5	<i>Cronartium coleosporioides</i>	Cronartiaceae	branch rust	Lodgepole
6	<i>Cronartium ribicola</i>	Cronartiaceae	aecial phoase	White pine
7	<i>Cronartium ribicola (?)</i>	Cronartiaceae	telial phase	Castilleja sp.
8	<i>Cryptoporus volvatus</i>	Polyporaceae	brown saprot fungus	Red Fir
9	<i>Fomitopsis pinicola</i>	Fomitopsidaceae	brown cubical decomposer	Red Fir
10	<i>Ganoderma lucidum</i>	Ganodermataceae	white rot decomposer	Red Fir
11	<i>Gymnosporangium libocedri</i>	Pucciniaceae	branch rust	Incense Cedar
12	<i>Herpotrichia nigra</i>	Lophiostomataceae	Snow mold	Ponderosa, Lodgepole
13	<i>Heterobasidion occidentale</i>	Bondarzewiaceae	white rot decomposer	Red Fir
14	<i>Hypholoma fasciculare</i>	Strophariaceae	wood saprophyte	Red Fir
15	<i>Luchnellula sp.</i>	Hyaloscyphaceae	branch canker	Red Fir
16	<i>Laetiporus sulphureus</i>	Polyporaceae	brown cubical decomposer	Red Fir
17	<i>Laricifomes officinalis</i>	Fomitopsidaceae	brown cubical decomposer	Larch
18	<i>Marssonina populi</i>	Incertae sedis	leaf blotch, anthracnose	Quaking aspen
19	<i>Melampusora occidentalis</i>	Melampsoraceae	leaf rust	Poplar
20	<i>Melampsorella caryophyllacearum</i>	Pucciniastreaeae	broom rust	Red Fir
21	<i>Meliola sp.</i>	Meliolaceae	black sooty mold	Alnus
22	<i>Neolentinus lepideus</i>	Gleophyllaceae	white rot decomposer	Red Fir
23	<i>Neonectria sp.</i>	Nectariaceae	branch canker	Alnus
24	<i>Oligoporus amarus</i>	Polyporaceae	brown cubical decomposer	Incense Cedar
25	<i>Oligoporus leucospongia</i>	Polyporaceae	brown cubical decomposer	Ponderosa, Lodgepole
26	<i>Omphalina sp.</i>	Tricholomataceae	wood saprophyte	Red Fir
27	<i>Phaeolus schweinitzii</i>	Polyporaceae	root and butt brown cubical decay	conifers
28	<i>Phellinus pini</i>	Hymenochaetaceae	white pocket rot decomposer	Red Fir
29	<i>Pholiota adipose</i>	Strophariaceae	sap-rot	Red Fir
30	<i>Pluteus cervinus</i>	Pluteaceae	lignicolus decomposer	wood
31	<i>Septoria sp.</i>	Mycosphaerellaceae	leaf spot and stem canker	Poplar
32	<i>Stereum sp.</i>	Stereaceae	sapwood decay	Alnus
33	<i>Trichaptum abietinum</i>	Polyporaceae	honey-comb sap-rot	Red Fir
34	<i>Phellinus termulae</i>	Hymenochaetaceae	white trunk rot	Quaking aspen



Photo 4. *Melampsorella caryophyllacearum* rust-broom (Left) and aecia stage (Center). Fruiting by *Laetiporus sulphureus* on fir (Right).



Photo 5. *Oligoporus leucospongia* on pine (Left). *Arceuthobium abietinum* on red fir (Right).



Photo 6. Uredial stage of *Melampsora* on poplar (Left). Incense cedar limb swelling caused by *Gymnosporangium libocedri* rust (Right).



2012 WIFWC BUSINESS MEETING MINUTES

Secretary – John Browning

The WIFDWC 2012 business meeting was called to order by Chairmen Alex Woods at 8:45 on 10/12/2012.

OLD BUSINESS

Holly Kearns presented the WIFDWC treasures report. The Treasurer's report is included as a separate document in the proceedings. For this year's WIFDWC there were over 90 registrants. Holly expressed concerns about WIFDWC tax status. Holly would like to hire a tax consultant to be sure that the WIFDWC tax situation is being handled properly. Brian Giles moved that the Treasurer hire a tax consultant. This was seconded by Bill Jacobi. The motion passed.

Ellen Goheen nominated Richard Reich as chairmen and Kristen Chadwick as secretary for the 2013 WIFDWC. The motion was seconded by MaryLou Fairweather. Motion passed. Richard announced that Blakey Lockman agreed to be program chair.

The 2013 WIFDWC is going to be held in Waterton Lakes National Park in Alberta, Canada. Tod Ramsfield has agreed to be in charge of local arrangements. The dates of the meeting will be from 10/7/2013 to 10/11/2013. Brian Giles suggested that a possible interesting pre-meeting field trip would be though the Sweet Grass Hills in Montana. Blakely Lockman agreed to research this possibility.

The 2014 WIFDWC will be in Logan Utah with Fred Baker as the local arrangements coordinator. Fred was unable to attend the business meeting but gave Alex two proposals concerning timing of this meeting to discuss. The IUFRO World Congress is in Salt Lake City from October 5-11. Fred's proposals are to have WIFDWC meet before or after the IUFRO meeting. The first proposal would have WIFDWC start around October 1st, this date maybe a problem for federal employees because this is right at the start of the fiscal year. The second proposal would be to have WIFDWC from 10/14 to 10/17. Fred's concern with the second proposal is the weather is more likely to deteriorate later in October. Brian Giles points out that there are probably field trips associated with the IUFRO meeting happening both before and after the meeting. Dave

Shaw moved that WIFDWC meet after the IUFRO Congress. Brian Giles seconded the motion and the motion passed.

Presentation from students who won the "Student Travel Award" was discussed. Bill Jacobi points out that there was no place in this year's program for travel award winners to present and moves that an hour be reserved on the program for these presentations in the future. Dave Shaw seconded the motion. It was discussed that these presentations could be a separate session or they could be blended in with other contributed paper sessions. The question was raised whether this would require an amendment to the bylaws to say that students winning the travel award need to make a presentation. Bill Jacobi suggests that Stefan Zeglen and the Student Travel Award Committee get together and draft the language for such an amendment. Stefan suggested that the motion be changed to recommend to the program chair that students who win the travel award be given time on the program for a presentation. This motion was seconded by Dave Shaw and the motion passed.

Joint national meeting with other forest pathology working groups was discussed. We would probably host it. This was discussed last year and Pete Angwin contacted the other groups and concluded that there was not great interest in such a meeting. If it were to happen WIFDWC would probably need to host it. This was discussed at last year's business meeting and tabled until 2013.

Location of the 2015 WIFDWC was the next item discussed. Both Mount Saint Helens, Washington and Newport, Oregon were suggested but rejected because Region 6 had just hosted a WIFDWC meeting last year. Alaska was suggested as a possible location. Robin Mulvey expressed concern over the Alaska weather in October. Concern was also expressed about the difficulty of getting to Alaska. To get around the October weather, Robin moved that WIFDWC meet in

Alaska in the spring of 2016. Dave Shaw seconded the motion. The motion passed.

This would mean that there would be no WIFDWC meeting in 2015. There could be a second meeting in the fall of 2016. The Canadians pointed out that two meetings in one fiscal year would be difficult unless one of the meetings was in Canada. Stefan Zeglen moved that the fall meeting for 2016 meeting held in BC. Robin Mulvey seconded the motion and the motion passed.

NEW BUSINESS

John Schwandt pointed out that it is WIFDWC tradition to have a moment of silence for deceased members. It was discussed that we did not have a moment of silence this year because we had a tribute for these members at the banquet. It was suggested that WIFDWC continue either the tribute or the moment of silence.

It was brought up that since WIFDWC has moved away from regional reports at the meetings that job openings are not being announced. There was a job board at the poster session at this meeting but there was some concern that it was not well utilized. Richard Reich suggests that WIFDWC have an electronic form for forest pathology jobs. Pete Angwin suggested we should give time for people to announce jobs, maybe during the poster session. Bill Jacobi moved interim reports be put in a short standard format and published in the proceedings. Stefan Zeglen pointed out that this would make the proceedings very thick. Ellen Goheen suggested that the proceedings are not timely enough for job announcements. Stefan suggested Regional reports be written up and handed out at meeting or posted on the WIFDWC web site. Bill Jacobi moved

that the WIFDWC secretary should request reports from the membership in a standard format prior to the meeting. Richard Reich seconded the motion and the motion passed.

Holding another joint meeting with the entomologist was discussed. It was suggested that the 2016 meeting in BC might be a good time to do this. Kristen Chadwick pointed out that one problem with this might be that the US Forest Service limits the number of employees that can attend a given meeting. Stefan Zeglen moved that we suggest to the fall 2016 organizing committee that they consider having a joint meeting with entomologists. Bill Jacobi seconded the motion and the motion passed.

Bill Jacobi moved to nominate Mike Cruickshank as the Canadian representative for the Outstanding Achievement Award Committee to replace Harry Kope who is rotating off the committee. Ellen Goheen seconded the motion and the motion passed.

It was brought up that the Student Travel Awards Committee also needs new people to join. Both John Schwandt and Blakely Lockman are going off the committee. Both Alex Woods and Robin Mulvey moved to nominate themselves. This was seconded by Richard Reich and was passed. Stefan moved to thank Blakely and John for their service on the committee.

Bill Jacobi moved to thank the organizing committee and those who took care of this year's local arrangements for the 2012 WIFDWC meeting.

The WIFDWC 2012 Business meeting was adjourned at 10am.

TREASURER'S REPORT, 60TH WIFDWC

TREASURER- Holly Kearns

The 60th annual WIFDWC had a large turnout at Lake Tahoe with 92 total registrants including 63 regular members, 15 students, 4 retirees, 3 single-day attendees, and 7 guests. The following is a summary of transactions for the WIFDWC accounts from 12/16/2011 through 12/31/2012. The WIFDWC Federal Tax Identification Number is available upon request.

	Income/	Balance	Total
All WIFDWC Accounts balance 12/16/11			\$21,715.33
WIFDWC Meeting Account balance 12/16/11		\$14,274.37	
Total registration	21,646.80		
Hotel meeting rooms, meals & breaks (total expense \$-2,413, \$1,087 refunded from \$3,500 deposit paid in 2011)	1,087.00		
Field trip transportation	-2,378.35		
Field trip supplies and lunches	-693.98		
Souvenirs and awards	-660.61		
Speaker expenses	-1,000.00		
Misc. meeting supplies	-28.76		
Interest	0.93		
2011 Proceedings (printing and formatting)	-3,505.53		
Deposit for 2013 Waterton Lakes Meeting	-2,100.00		
Sales of proceedings	75.00		
Estimated Net Proceeds 60 th WIFDWC (\$10,972.10 incl. \$ -3,500 est. for Lake Tahoe proceedings)			
WIFDWC Meeting Account balance 12/31/12		\$26,716.87	
Hazard Tree Committee Account balance 12/16/11		\$5,401.96	
Reimbursement of 2010 pre-ceedings expenses – J. Glaeser	-910.40		
Deposit for 2013 Sedona Meeting	-2,019.41		
Hazard Tree Committee Account balance 12/31/12		\$2,472.15	
Student Travel Award Fund balance 12/16/11		\$2,039.00	
2012 Student Travel Awards	-1,600.00		
2012 Silent Auction Proceeds	1,190.00		
Student Travel Award Fund balance 12/31/12		\$1,629.00	
International Sponsorship Fund balance 12/16/11		\$0.00	
Grant to sponsor four invited forest pathologists	20,000.00		
Conference and travel expenses for four sponsored pathologists from Guam and Mexico	-10,616.00		
International Sponsorship Fund balance 12/31/12		\$9,384.00	
All WIFDWC Accounts balance 12/31/12			\$40,202.02



BYLAWS OF THE WESTERN INTERNATIONAL FOREST DISEASE WORK CONFERENCE
Passed by a vote of the Membership at the Business Meeting of October 13, 2011

Article I

Objectives _____

The Western International Forest Disease Work Conference (WIFDWC) was formed in 1953 to provide a forum for information exchange among forest pathologists in western North America. The primary objectives of the organization are:

- To exchange information on forest pests and related matters through periodic meetings and other appropriate means,
- To promote education, research and extension activities in forest pathology, and
- To sustain and improve the health of western North America's forests.

Article 2

Membership _____

Membership is open to individuals who are engaged in forest pathology related endeavors in western North America. These include but are not limited to: research, survey, management, teaching or extension activities pertaining to tree diseases, forest health, or deterioration of forest products.

Western North America is defined as Canada: British Columbia, Yukon, Alberta, Manitoba, Saskatchewan; United States: Washington, Oregon, California, Idaho, Nevada, Utah, Arizona, Montana, Wyoming, Colorado, New Mexico, North Dakota, South Dakota, Nebraska, Kansas, Alaska, Hawaii, Guam, the Commonwealth of the Northern Mariana Islands and other Pacific Islands in Micronesia; and all of Mexico.

Membership is established after attending one Western International Forest Disease Work Conference. Members must attend another Western International Forest Disease Work Conference within 5 years or their membership is no longer valid.

Honorary Life membership will be automatically awarded to those members of WIFDWC (as defined above) who have attend at least 5 previous meetings of WIFDWC and have retired. Newly retired members who meet these criteria should notify the current WIFDWC Secretary of their status. Other members who have retired but do not meet the attendance criteria or other outstanding contributors to the field of Forest Pathology may request, or be proposed for, Honorary Life Membership by members present at an annual business meeting.

A list of Honorary Life Members will be published in the Proceedings of each meeting.

A 50 percent or more reduction in the registration fees for Honorary Life Members, to include a copy of the Proceedings, should be considered by the Executive Committee, as per Article 7.

Article 3

Officers _____

WIFDWC officers will include a Conference Chairperson, Secretary, Treasurer, Program Chairperson, Historian and Web Coordinator. The Conference Chairperson and Secretary will be elected by majority vote of the membership at the annual business meeting. If there is no majority, an acting Chairperson will be appointed by the current Conference Chairperson. The tenure of the Conference Chairperson and Secretary begins at the conclusion of the WIFDWC meeting where they were elected and ends when all business from the next WIFDWC is completed. The Treasurer, Historian and Webmaster will be elected every five years, to serve for the following 5 years.

Duties of the Conference Chairperson

At each WIFDWC, the Conference Chairperson will run the general and business meetings. The Conference Chairperson will appoint an interim Program Chairperson at the start of each WIFDWC to gather suggestions and opinions to guide the conference in the planning of next year's conference. The Conference Chairperson will also appoint three members to serve as the "railroad committee" to nominate candidates for next year's Conference Chairperson and Secretary (and every fifth year, Treasurer, Historian and Web Coordinator). The Conference Chairperson may appoint members to assist in conducting the affairs of the Conference including, but not limited, to Local Arrangements representative(s) and Program Chairperson. The Conference Chairperson may also appoint ad hoc committees and their chairpersonss as deemed necessary to assist in carrying out the mission of WIFDWC.

In the event that a new Conference Chairperson cannot carry out their duties, the previous Chairperson will carry them out. If another member of the Executive Committee cannot or will not carry out their duties the Conference Chairperson may appoint a replacement.

Duties of the Secretary

The Secretary shall maintain the membership and mailing lists. The Secretary shall send out meeting notices to the membership, take minutes at the business meeting, and compile and distribute the Conference proceedings.

The secretary will query all Honorary Life Members to determine if they want to receive a free copy of the proceedings and only those responding in the affirmative will receive a copy.

Duties of the Treasurer

The Treasurer shall receive all payments, be custodian of WIFDWC funds, keep an account of all moneys received and expended, and make commitments and disbursements authorized by the Conference Chairperson. At the annual business meeting the Treasurer shall make a report covering the financial affairs of WIFDWC. All funds, records and vouchers in the Treasurer's control should be subject to inspection by the Executive Committee.

Duties of the Program Chairperson

The Program Chairperson is appointed by the Conference Chairperson. The Program Chairperson is responsible for all aspects of the conference agenda including arranging the format and timing of the meeting, selecting panel chairpersons or moderators, selecting the poster session coordinator, assigning subject matter committee meeting times, and arranging keynote, contributing paper and other speakers.

Duties of the Historian

The Historian will keep a complete set of WIFDWC proceedings and answer any inquires as needed. The Historian will contact the WIFDWC Secretary and provide the address for mailing the archival copy of the proceedings.

Duties of the Web Coordinator

The Web Coordinator will create and manage the WIFDWC website. The Web Coordinator will supervise the hosting, security and access of the website. Content for the website will be provided by the Executive Committee for each meeting. The Web Coordinator will ensure that previous WIFDWC meeting websites and their proceedings are archived and linked to the current website.

Compensation

Officers will not be compensated for their services.

Non-liability of Officers

The officers shall not be personally liable for the debts, liabilities or other obligations of the WIFDWC.

Article 4

Decision Making Process _____

The business meeting will be run under Roberts Rules of Order. Meetings are open to the public and non-members may participate in meetings. Only members may vote.

Decisions will be made by majority, with each member granted one vote. Votes may be called for at the annual business meeting or via electronic ballot (i.e., e-mail ballot, web poll, etc.). A quorum is reached when more than 25 members are present.

Article 5

Finances _____

Expenditures

The Conference Chairperson may authorize expenditures of WIFDWC funds. Standing Committee Chairs may similarly authorize the expenditure of funds that are generated by their standing committees (e.g., Hazard Trees Committee). Checks, orders for payment, etc. may be signed by the Treasurer, or other person designated by the Chairperson. The Executive Committee may determine which and how many outside speakers they want to invite, and travel costs for such speakers can be paid from registration fees.

Contracts

The Conference Chairperson may authorize any officer or agent of WIFDWC to enter into a contract on behalf of WIFDWC. Standing Committee Chairs may similarly authorize any agent of their standing committee to enter into a contract on behalf of their committee. Unless so authorized, no person shall have any authority to bind WIFDWC or any standing committee to any contract.

Gifts

The Conference Chairperson or the Treasurer may accept on behalf of the WIFDWC any contribution, gift, or bequest. Commercial sponsorship of conference special events is not allowed.

Fiscal year

The WIFDWC fiscal year shall begin on the first of January and end on the last day of December.

Article 6

Bylaws _____

Amendments

Changes to bylaws shall be made available to all WIFDWC members for review at least one month prior to the next business meeting. A two-thirds majority is required to pass a motion to amend existing bylaws if the vote is held at a business meeting. An affirmative vote from at least 26 members is required to approve a motion voted on by electronic balloting (i.e., e-mail ballot, web poll, etc.).

Article 7

Meetings _____

Frequency

The WIFDWC endorses holding annual meetings but will, on vote of the membership, change the time of any particular meeting when circumstances dictate that such action be taken.

Date

WIFDWC endorses holding meetings in late summer but will change the interval between any two meetings when circumstances dictate that such an action be taken. Meeting dates will be set by the Executive Committee for each meeting.

Registration

Registration will be reduced by half, if possible, for graduate students and Honorary Life Members. It will be at the discretion of the WIFDWC Executive Committee for each meeting to offer a further reduction in fees to graduate students and Honorary Life Members and to offer further reduced fees to others such as retired professionals and visitors.

Article 8

Committees _____

There shall be two types of committees, namely

- a) Standing Committees – as designated in the by-laws, and
- b) Ad Hoc Committees – as appointed by the Conference Chairperson to serve for a term specified by the Chairperson.

The chair of each standing committee shall prepare a report of the committee activities for the membership. The report will be submitted by the publication deadline to the Secretary for inclusion in the proceedings.

The following are WIFDWC standing committees:

- Executive Committee
 - Composed of the elected Conference Chairperson, Secretary, Treasurer, Historian and Web Coordinator.
 - The Conference Chairperson may appoint a Program Chair, Local Arrangements representative(s) and other persons as necessary to carry out the business of the next WIFDWC meeting.
 - The Executive Committee may invite non-member speakers to the annual meeting and pay their travel expenses from conference registration fees.
- Awards Committee
 - Composed of three members with the longest serving member designated as chair.
 - Committee will be comprised of a representative from each of the following – a university employee, a public agency employee, and one member at large. At least one member should be from Canada.
 - The chair's term will be completed at the end of the annual business meeting and a new junior member will be appointed by the Conference Chairperson. The most senior serving member will assume the chair for the next year.
 - The chair will provide a report of activities at the annual business meeting.
 - Responsible for accepting and evaluating nominations and determining recipients of the WIFDWC Outstanding Achievement Award as outlined in Article 10.
- Student Scholarship Committee
 - Composed of four members with the longest serving member designated as chair.
 - The chair will provide a report of activities at the annual business meeting.
 - The committee will be comprised of at least one representative from a university.
 - Replacement of committee members will be by election at the annual business meeting.
 - The committee is responsible for fundraising to finance any awards given by the committee.
 - The committee is responsible for determining and advertising the award application criteria, receiving and evaluating applications and determining recipients of the WIFDWC Student Travel Awards as outlined in Article 10.

- Hazard Trees Committee,
- Dwarf Mistletoe Committee,
- Root Disease Committee,
- Rust Committee,
- ~~Disease Control Committee~~ [disbanded 2002],
- Nursery Pathology Committee [approved 2002],
- Foliage and Twig Diseases Committee [established 2007, approved 2009],
- Climate Change Committee [established 2007, approved 2010].

Ad hoc committees are established by the Conference Chairperson to carry out various functional needs (e.g., the annual Nominating Committee). Ad hoc committees carry out specific, normally short term, tasks required by the membership. The terms of reference for ad hoc committees will be determined by the Conference Chairperson in consultation with the membership.

Article 9

Proceedings_____

Papers for each year's proceedings must be submitted to the Secretary by the deadline set for each conference by the Secretary.

Distribution of proceedings is made to all paid registrants and honorary members who have indicated a desire to receive them and will be made available to others at cost.

Article 10

Awards_____

Outstanding Achievement Award

Members may recognize outstanding achievement in the field of forest pathology by bestowing the WIFDWC Outstanding Achievement Award. The award will recognize an individual that has, in the opinion of the membership, contributed significantly to the field of forest pathology in western North America.

The award will be presented during the conference by the chair of the Awards Committee or designate. The recipient will receive a framed certificate or plaque. The recipient will present a keynote address at the following year's WIFDWC. A list of recipients will be published in the proceedings.

Members may nominate other current or active members for the award; they may not nominate themselves. A member may only make one nomination each year. A nomination must include: a short introductory letter, a narrative of the nominee's qualifications, educational background, work history, etc., letters of support from other members and organizations, and copies of a few of the nominee's published works. Nominations are due no later than three months prior to the start of next year's conference and must be sent to the Awards Committee chair.

The Awards Committee may decide to not make an award if no suitable candidates are nominated.

Student Travel Awards

Members encourage participation in the annual conference by students engaged in studies in the field of forest pathology by bestowing the WIFDWC Student Travel Awards to enable their attendance. The awards are intended for students currently enrolled in a university graduate level program with a thesis or dissertation topic relevant to the field of forest pathology. The awards are intended to assist with conference-related expenses.

Criteria for application and selection of award recipients will be determined by the committee and made public at least four months prior to the early registration date for the meeting or by the first WIFDWC mailing. Completed applications are due by the deadline set by the committee.

The awards will be presented at least four weeks prior to the early registration date for the conference by the chair of the committee or designate. The recipients will receive an award of up to US\$500 depending on funding availability. Recipients will be required to make an oral or poster presentation at the meeting for which they received the award. Oral presentations are preferred.

The committee may decide to not make an award if no suitable candidates apply.

Select Motions and Decisions_____

1998

Outstanding Achievement Award—established.

1999

Honorary Life Members—members added and provisions discussed (see 1996 Proceedings for historic retrospective on HLM).

Assisting Outside Speakers—amendment passed.

Website—Committee Reports and Meeting synopsis by the Chairperson would be posted; web committee (Baker, Muir, and Adams) formed.

2000

Outstanding Achievement Award—staggered committee established and recommendations made.

Joint Meetings with WFIWC—motions passed to meet in 2004, have dual program chairs, form a planning committee in 2001 for the joint meeting.

2001

Standing Committees—proposal to reorganize Disease Control Committee tabled.

2002

Standing Committees—motion passed to disband the Disease Control Committee and establish a Nursery Pathology Committee.

2004

Outstanding Achievement Award—changes to the Bylaws for this award were proposed and accepted by the membership.

Executive Committee—motion to make Webmaster an official position on the committee was approved.

2007

Standing Committees—motion passed to create both an ad hoc Foliar and Shoot Diseases Committee and a Climate Change Committee.

2008

Digital Proceedings—motion to make WIFDWC proceedings available on the website was approved.

2009

Standing Committees—motion passed to confirm the Foliage and Twig Diseases Committee as a standing committee.

2010

Standing Committees—motion passed to confirm the Climate Change Committee as a standing committee.

Fund Raising—the first WIFDWC Silent Auction was held to raise funds for graduate student travel awards.

2011

Standing Committees—motion passed to add the Student Scholarship Committee as a standing committee.

Business Meeting—motion passed outlining requirements needed to pass a motion by means of an electronic ballot.

Bylaw items changed during the October 13, 2012 revision:

- Clarification of terminology and titles;
- Inclusion of option for electronic balloting;
- Clarification of WIFDWC officers duties and responsibilities;
- Updating of committees structure;
- Formalization of Outstanding Achievement Award and Student Travel Awards; and
- Allowing stand committee chairs to expend funds and enter into contracts.



WIFDWC OUTSTANDING ACHIEVEMENT AWARD RECIPIENTS

Year	Recipient	Meeting	Comments
2000	Lew Roth	Kailua-Kona, HI	For pioneering work on <i>Phytophthora lateralis</i> , <i>Armillaria</i> and dwarf mistletoes, and for inspiration and leadership of a generation of plant pathology students and colleagues.
2000	Duncan Morrison	Kailua-Kona, HI	For long-standing contributions to forest pathology research, especially in relation to roots diseases and tree hazards.
2001	Bob Gilbertson	Carmel, CA	For contributions to the taxonomy and identification of wood-inhabiting basidiomycete fungi.
2003	Everett Hansen.	Grants Pass, OR	For strong leadership in forest pathology including research on the biology and management of tree and seedling diseases of western conifers
2004	Bob James	San Diego, CA	For strong leadership in forest pathology especially technology transfer and research on the biology and management of forest nursery diseases for growers and nursery pathologists throughout the West.
2005	Walt Thies	Jackson, WY	For sustained long-term high quality research on laminated root rot and other root diseases of forest trees.
2006	Bart van der Kamp	Smithers, BC	In recognition of outstanding lifetime contribution to tree disease research and for inspiring a generation of students and colleagues in the field of forest pathology.
2006	Alan Kanaskie	Smithers, BC	For outstanding leadership, as a practicing forest pathologist, in the management of Swiss Needle Cast and Sudden Oak Death.
2007	Richard Hunt	Sedona, AZ	In recognition of his valuable research and extension efforts on white pine blister rust, along with many other contributions to forest pathology and biology.
2009	Bill Jacobi	Durango, CO	In recognition of your 30-plus years as an educator, researcher, organizer, advocate and practitioner of forest pathology.
2009	Bob Edmonds	Durango, CO	In recognition of your 40-plus years as, an educator, researcher, organizer, advocate and practitioner of forest pathology and ecology.
2010	Paul Hennon	Valemount, BC	For sustained, significant contributions to our knowledge and understanding of forest disease dynamics and ecology.
2011	Susan Frankel Ellen Goheen	Leavenworth, WA	For leadership in the science and practice of forest pathology and for critical contributions to the management of Sudden Oak Death.
2012	John Schwandt	Tahoe City, CA	In recognition of career long contributions to forest pathology, particularly work with white pine blister rust. And for collaborations with natural resource professionals, community involvement, tutoring and advocating for young people, and promoting good urban forestry practices.

No award given for years 2002 & 2008.

OAA COMMITTEE MEMBERS

Year	Chair	Members	
2000	J. Byler	W. Littke	B. van der Kamp
2001	W. Littke	B. van der	R. Sturrock
2002	B. van der Kamp	R. Sturrock	G. Filip
2003	R. Sturrock	G. Filip	
2004	G. Filip	D. Goheen	S. Zeglen
2005	D. Goheen	S. Zeglen	D. Shaw
2006	S. Zeglen	D. Shaw	B. Ferguson
2007	D. Shaw	B. Ferguson	R. Reich
2008	B. Ferguson	R. Reich	E. Goheen
2009	R. Reich	E. Goheen	P. Angwin
2010	E. Goheen	P. Angwin	H. Kope
2011	P. Angwin	H. Kope	B. Jacobi
2012	H. Kope	B. Jacobi	P. Hennon
2013	B. Jacobi	P. Hennon	M. Cruickshank

STANDING COMMITTEES AND CHAIRS, 1994-2012

Committee	Chairperson	Term
Hazard Trees	J. Pronos	1994—2005
	P. Angwin	2006—present
Dwarf Mistletoe	R. Mathiasen	1994—2000
	K. Marshall	2001—2003
	F. Baker	2004—present
Root Disease	G. Filip	1994—1995
	E. Michaels Goheen	1996—2005
	B. Ferguson	2006—2009
	M. Cleary	2010—2011
	B. Lockman	2012
Rust	J. Schwandt	1994, 2005
	R. Hunt	1995—2004
	H. Kearns	2006—2011
	H. Maffei	2012
Disease Control ^a	B. James	1995—2002
Nursery Pathology	B. James	2002—2005
	K. Mallams	2007—2010
	W. Littke	2011—present
Foliar and Twig Diseases ^b	H. Kope	2007—present
Climate Change ^c	S. Frankel	2007—2008
	S. Frankel & D. Shaw	2009—present

^aDisease Control committee was disbanded in 2002.

^bFoliar and Twig Diseases committee was made full charter member in 2009.

^cClimate Change committee was made full charter member in 2010.



PAST ANNUAL MEETING LOCATIONS AND OFFICERS
Meetings and Officers, 1953—2012

Annual	Year	Location	Chairperson	Secretary-Treasurer	Program Chair	Local Arrangements
1	1953	Victoria, BC	R. Foster			
2	1954	Berkeley, CA	W. Wagener	P. Lightle		
3	1955	Spokane, WA	V. Nordin	C. Leaphart	G. Thomas	
4	1956	El Paso, TX	L. Gill	R. Davidson	V. Nordin	
5	1957	Salem, OR	G. Thomas	T. Childs	R. Gilbertson	
6	1958	Vancouver, BC	J. Kimmey	H. Offord	A. Parker	
7	1959	Pullman, WA	H. Offord	R. Foster	C. Shaw	
8	1960	Centralia, WA	A. Parker	F. Hawksworth	J. Parmeter	K. Shea
9	1961	Banff, AB	F.Hawksworth	J. Parmeter	A. Molnar	G. Thomas
10	1962	Victoria, BC	J. Parmeter	C. Shaw	K. Shea	R. McMinn
11	1963	Jackson, WY	C. Shaw	J. Bier	R. Scharpf	L. Farmer
12	1964	Berkeley, CA	K. Shea	R. Scharpf	C. Leaphart	H. Offord
13	1965	Kelowna, BC	J. Bier	H. Whitney	R. Bega	A. Molnar
14	1966	Bend, OR	C. Leaphart	D. Graham	G. Pentland	D. Graham
15	1967	Santa Fe, NM	A. Molnar	E. Wicker	L. Weir	P. Lightle
16	1968	Couer D'Alene, ID	S. Andrews	R. McMinn	J. Stewart	C. Leaphart
17	1969	Olympia, WA	G. Wallis	R. Gilbertson	F.Hawksworth	K. Russell
18	1970	Harrison Hot Springs, BC	R. Scharpf	H. Toko	A. Harvey	J. Roff
19	1971	Medford, OR	J. Baranyay	D. Graham	R. Smith	H. Bynum
20	1972	Victoria, BC	P. Lightle	A. McCain	L. Weir	D. Morrison
21	1973	Estes Park, CO	E. Wicker	R. Loomis	R. Gilbertson	J. Laut
22	1974	Monterey, CA	R. Bega	D. Hocking	J. Parmeter	
23	1975	Missoula, MT	H. Whitney	J. Byler	E. Wicker	O. Dooling
24	1976	Coos Bay, OR	L. Roth	K. Russell	L. Weir	J. Hadfield
25	1977	Victoria, BC	D. Graham	J. Laut	E. Nelson	W. Bloomberg
26	1978	Tucson, AZ	R. Smith	D. Drummond	L. Weir	R.Gilbertson
27	1979	Salem, OR	T. Laurent	T. Hinds	B. van der Kamp	L. Weir
28	1980	Pingree Park, CO	R. Gilbertson	O. Dooling	J. Laut	M. Schomaker
29	1981	Vernon, BC	L. Weir	C.G. Shaw III	J. Schwandt	D. Morrison R. Hunt
30	1982	Fallen Leaf Lake, CA	W. Bloomberg	W. Jacobi	E. Hansen	F. Cobb J. Parmeter
31	1983	Coeur d'Alene, ID	J. Laut	S. Dubreuil	D. Johnson	J. Schwandt J. Byler
32	1984	Taos, NM	T. Hinds	R. Hunt	J. Byler	J. Beatty E. Wood
33	1985	Olympia, WA	F. Cobb	W. Thies	R. Edmonds	K. Russell
34	1986	Juneau, AK	K. Russell	S. Cooley	J. Laut	C.G. Shaw III
35	1987	Nanaimo, BC	J. Muir	G. DeNitto	J. Beatty	J. Kumi
36	1988	Park City, UT	J. Byler	B. van der Kamp	J. Pronos	F. Baker
37	1989	Bend, OR	D. Goheen	R. James	E. Hansen	A. Kanaskie

Meetings and Officers, 1953-2012 (cont.)

Annual	Year	Location	Chair-person	Secretary	Treasurer	Program Chair	Local Arrangements	Historian	Web Coordinator
38	1990	Redding, CA	R. Hunt	J.Hoffman	K. Russell	M.Marosy	G. DeNitto		
39	1991	Vernon, BC	A. McCain	J. Muir	K. Russell	R. Hunt	H. Merler		
40	1992	Durango, CO	D. Morrison	S. Frankel	K. Russell	C.G. Shaw III	P. Angwin		
41	1993	Boise, ID	W. Littke	J. Allison	K. Russell	F. Baker	J. Hoffman		
42	1994	Albuquerque, NM	C.G. Shaw III	G. Filip	K. Russell	M. Schultz	D. Conklin T. Rodgers		
43	1995	Whitefish, MT	S. Frankel	R. Mathiasen	K. Russell	R. Mathiasen	J. Taylor J. Schwandt		
44	1996	Hood River, OR	J. Kliejunas	J. Beatty	J. Schwandt	S. Campbell	J. Beatty K. Russel		
45	1997	Prince George, BC	W. Thies	R. Sturrock	J. Schwandt	K. Lewis	R. Reich K. Lewis		
46	1998	Reno, NV	B. Edmonds	L. Trummer	J. Schwandt	G. Filip	J. Hoffman J. Guyon	D. Morrison	
47	1999	Breckenridge, CO	F. Baker	E. Michaels Goheen	J. Schwandt	J. Taylor	D. Johnson		
48	2000	Waikoloa, HI	W. Jacobi	P. Angwin	J. Schwandt	S. Hagle	J. Beatty		
49	2001	Carmel, CA	D. Johnson	K.Marshall	J. Schwandt	A. Kanaskie	S. Frankel		
50	2002	Powell River, BC	B. van der Kamp	H. Maffei	J. Schwandt	P. Hennon	S. Zeglen R. Diprose		
51	2003	Grants Pass, OR	E. Hansen	B. Geils	J. Schwandt	H. Merler	E. Michaels Goheen		
52	2004	San Diego, CA	E. Goheen	B. Lockman	J. Schwandt	H. Merler K. Lesiw	J. Pronos J. Kliejunas S. Smith		
53	2005	Jackson, WY	M. Fairweather	H. Merler J. Guyon	J. Schwandt	K. Burns	J. Hoffman F. Baker J. Guyon		
54	2006	Smithers, BC	K. Lewis	M. Jackson	J. Schwandt	B. Lockman	A. Woods		
55	2007	Sedona, AZ	S. Zeglen	M. McWilliams	J. Schwandt	J. Worrall	M.Fairweather B. Geils B. Mathiason		
56	2008	Missoula, MT	G. DeNitto	F. Baker	J. Schwandt	W. Littke	B. Lockman M. Jackson		
57	2009	Durango, CO	G. Filip	J. Adams	J. Schwandt	D. Shaw	K. Burns		
58	2010	Valemount, BC	R. Sturrock	M. Fairweather	J. Schwandt	D. Goheen	M. Cleary R. Reich		
59	2011	Leavenworth, WA	P. Angwin	S. Zeglen	H. Kearns	A. Kanaskie	G. Filip A. Saavedra A. Ramsey-Kroll D. Omdal	R. Sturrock	
60	2012	Tahoe City, CA	A. Woods	J. Browning	H. Kearns	P Hennon	P. Cannon B. Woodruff		J. Adams

Bylaws passed at 1998 WIFDWC Business Meeting identify officers as chairperson and secretary elected at annual business meeting and treasurer and historian, elected every five years.

IN MEMORIAM

A Reminiscence of Fields White Cobb, Jr., 1932–2011



The first time I heard the name *Fields Cobb* was in the summer of 1971, while working with a blister rust crew at Mountain Home in the southern Sierra Nevada. The crew consisted of Ron Wakimoto and Mark Schultz, recent graduates of the UC Berkeley Forestry School. Both of them, Ron in particular, were full of tales about Berkeley Forestry and the idiosyncrasies of the professors. However, their stories about Fields Cobb were by far the most memorable. It soon became apparent that Fields and his colleague Dick Parmeter were people I would have to meet some day. Ron told stories about Fields' teaching style and the varied anecdotes Fields used to make points about forest disease. Although I didn't know it then, I was being groomed for both a new career and a likely major professor, though that would not happen for another fifteen years. The stories Ron told about forest pathology at Berkeley, and in particular, about Fields' teaching methods, would stick with me until I finally experienced them first-hand. What was obvious, however, was that Fields was a dominant presence in forest pathology at Berkeley, both physically and mentally, and, whether a student came to love him or just tolerate him, he or she would never forget him.

In 1986, I took leave from the Forest Service and enrolled at Berkeley in a pathology PhD program. I bumped around for a bit trying to figure out which professor I would work with, until Fields one day volunteered that Bill Libby, Bro Kinloch, and he had just been funded for research on western gall rust, and he asked if I wanted to join Bill and him on a drive to Blodgett (a UCB field station near Georgetown) so we could discuss my interest in the project and I could get to know Bill. On the way up, Fields drove and told tall stories and Bill simultaneously reviewed a manuscript and bantered with us. As a result, Bill was willing to take me on, and so, later, was Bro; very shortly I was embarked on a PhD program with Fields as my major professor. It was another 14 years until I got back to the Forest Service again. My desk and lab bench were in a large "bullpen" that separated Dick's and Fields' offices on the west end of Giannini Hall. It was there that I really got to know Fields, for, whenever he was on campus, the students in the bullpen couldn't avoid him, his stories, or his booming voice. Thus I was drawn into the Cobb orbit.

Orbit is just the right word. The delightful and the difficult elements of Fields' personality were so finely balanced that one couldn't help but circle the Cobbian planet, trying to keep one's distance for fear of being overwhelmed by his personality and his occasionally ferocious rants, and simultaneously being drawn in by his wide-ranging intellect, his complex and entertaining stories – often about the Civil War, his infectious sense of humor, and his deep love for forests and forest pathology. Fortunately, he did not hold court at 131 Giannini every day, and thus one could catch a breath once in a while and get some reading or research or experimenting done; but when he was in the lab (although he had an office with a door, he preferred to sit at a table outside the office, in the bullpen, so that he would be right THERE among us), you couldn't help but be drawn in by his vivid, forcefully expressed thoughts, his jokes and laughter, and his serious conversations. During our thesis research meetings with Bro and Bill, I learned to sit back and listen, and let the three of them hash out whatever issues may have arisen, knowing that Fields would argue vociferously for my interests, and my weighing in would probably be fruitless. Fields stood up for his students, whether they appreciated it or not.

When Fields was on campus, it was as if you were in the midst of a tornado, a bustling town-square of ideas and conversation, and you either joined in or you escaped to the library; Fields was unavoidable, sometimes impossibly so, but mostly entertaining and always very insightful. This was the Socratic method refined to a homely and Cobbian essence, sometimes on topics that seemed to bear no relation to forests or pathology or your desire to get a

degree, but always memorable and ultimately an unforgettable and essential part of your education. I often wondered about those students who were primarily educated in class or at the lab bench, and never got to duke it out with Fields, the conversations ranging wildly but eventually always coming back to forest pathology, sometimes by circuitous and incomprehensible, but always entertaining, routes.

Fields was a hands-on, in-your-face teacher. He loved debate and forceful exchanges. He was passionately committed to the art of argumentation. And his verbal adversaries merited his respect if they gave back as good as they got, as long as their positions were intelligently and succinctly argued. If you were either dismissive of the art of argument or else refused to engage, he could become angry with you or else lose interest. If you honestly and forthrightly disagreed with Fields, he might become frustrated, but he would ultimately respect your opinion if it was energetically and honestly upheld. You just had to be ready to stand your ground when he backed you into a corner.

One aspect of Fields that most students might have missed was his understanding and appreciation of children and young adults. Perhaps that's one of the attributes of a great teacher, someone who can connect with people of all ages. My sons would occasionally come by the lab after school, and when Fields was there he would give them his complete attention. He never treated them as unequals or pandered to them; if I had to do something in the lab, I would occasionally glance over to see what was up and would find Fields in animated conversation with one or both, conversations that were apparently equally interesting to all. On a lighter side, there were the practical jokes. Once he had a tiny house that exploded if you tried to open the door (those of us who knew his ways would be wary of such things, for practical jokes were endlessly entertaining to Fields, mainly for the reactions they elicited in others.) My younger son, who was about 8 at the time, was initiated into the Cobb sense of humor by that trick house, and fortunately he loved the surprise. He'd ask Fields to set it back up after each explosion, and neither of them failed to tire of setting it off again and again. Those who never witnessed, or perhaps couldn't appreciate, this aspect of Fields would have missed something very special: a child-like enthusiasm for pure, silly fun.

Fields was immensely proud of his ancestry as a Virginian, born in an old wooden house in the small town of Dendron, across the James River from where Pocahontas had lived. His Virginia roots were deep in him, and he cherished the saddle that General Robert E. Lee had given his ancestor after the surrender at Appomattox, so that he could ride home on horseback, his honor intact. Fields loved to tell stories of Lee and Stonewall Jackson, and of growing up hunting and fishing in the woods around Dendron. He cherished his rural Virginia roots, yet he married a Yankee, taught at Berkeley, and was often politically out of step with his fellow-professors. During the height of the free-speech movement on the Berkeley campus, he went out among the protesting students and talked one-on-one with them, trying to learn for himself what the beliefs were that motivated their protests, supporting those that he found to be justified and arguing vociferously against those that he thought specious. Fields was a brave and generous man, and when he concluded that the actions of another were wrong, he would offer his opinions forcefully and honestly, which could make some students and faculty uncomfortable. As one of his Virginia cousins wrote to him when he was ill, "[Y]ou have always lived upholding the honor of your family and old Virginia. And you have demonstrated the greatest virtues of other great sons of Virginia – the truthfulness of George Washington, the faithfulness to and love of family seen in Robert E. Lee, and conviction to honoring the rights and beliefs of all people as exhibited by Thomas Jefferson. My father certainly loved to give you a hard time for espousing those 'liberal' ideas, but we all cherished your deep sense of fairness and insistence on always doing what is right."

But there was something more to Fields as well. This same cousin also wrote, "You have certainly added levity to our lives, helping us remember that good humor makes life more livable." His full-throated laughter — especially when he'd pulled some trick on you and you fell for it, hook, line, and sinker — was infectious, and his victim could not help but laugh too. And, if you were successful in pulling a trick on him? There would be a brief moment of hesitation as he caught on, and then a roar of appreciation as he acknowledged what had happened, and then admitted how he wished he'd thought of that. The license plates on his car read "RUFASA", which led the curious to imagine the driver was some exotic foreigner, at least until they learned that the owner's last name was "Cobb".

Ultimately, however, Fields was a great teacher, a job he took seriously, whether in the classroom or in the woods. At the Institute of Forest Genetics in Placerville, CA, there is a large ponderosa pine beside an old irrigation ditch west of the Eddy Arboretum. When the Institute was founded in 1925, there were few pines left after the Gold Rush, so this tree and its neighbors probably seeded onto the edge of the ditch after the gold and the miners, but not the water, had disappeared and most of the old forest with them. The tree is about 4 ft. across at the base today, with a large rust gall on the ditch side, away from the road. Fields and his colleague Joe Ogawa would bring a plant pathology class up from Davis every other year or so, to learn about forest diseases, and eventually they would end up at this tree, and Fields would ask if anyone knew what was wrong with it. Fields had done this often enough that he knew what would happen. At this point in the tour, most of the students were ready to get back to the tomato or sugar beet diseases they were studying down in the Sacramento Valley – *real* crops and *real* plant pathology – but Fields was insistent that they had to answer his question, and he wouldn't let them go until someone had finally climbed around the back side of the tree and yelled out “western gall rust”. Then everybody else had to do it too. It was, and still is, a gigantic gall, but until you get around to the back side of the tree you don't really comprehend that this is not a mechanical wound but the slow and incremental result of a pathogen that infected the tree when it was no more than a couple feet tall. But Fields insisted that they make that discovery themselves and, if they learned nothing else on that visit, they learned about gall rust, because Fields insisted they do all the work.

The last time I visited Fields and Tavie at their home in northern Idaho was in the summer of 2007 (the photo is of Fields wishing me good-bye on the porch). I stayed for three days, and the first day Fields took me down to his study in the basement, where all the unfinished projects were shelved, reams of paper devoted to studies that never were finished, some not even started. There was the saddle from General Lee in the middle of the room, and there was enough material on the shelves to jump-start a dozen or so PhD projects. We reluctantly agreed that neither of us would be doing anything about that any time soon, and then he and I headed to lunch at a lakeside restaurant a couple of miles down the road.

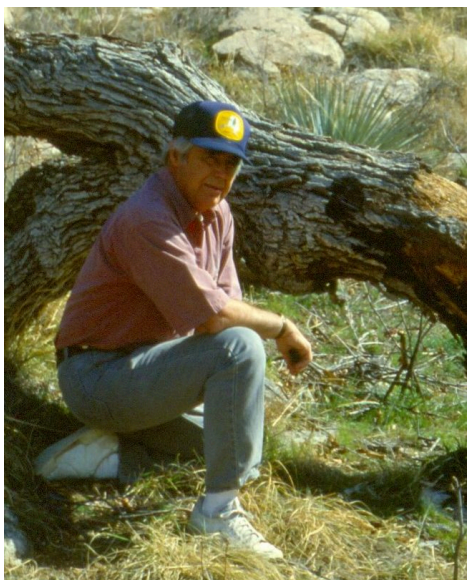
It was a lovely, sunny afternoon, and we were seated outside on the deck above Lake Pend Oreille. Fields had been tired all week, and Tavie had decided not to join us. Soon, the waitress, who was no older than 16 or so, came with the menus, and she and Fields got to talking. Before I knew it Fields was telling her stories and the waitress had gone from a somewhat skeptical teenager to apparently convinced that he was the most charming man she'd ever met. Pretty soon another waitress came out, and then another, and though we were the only patrons there, the restaurant became lively and boisterous and everyone was laughing at Fields' stories, especially Fields. Then the cook popped her head out to see what all the commotion was, and in a second or so Fields had charmed her as well. We soon got our food and began to eat, but throughout the meal various people, including the owner, emerged from inside and came out to meet Fields, and within seconds of arriving were doubled-over laughing at something he had said. Fields swore he'd never been there before, which I doubted, but it may be true. When Fields was charming, he was the most charming person I've ever known.

I miss him.

Detlev Vogler

IN MEMORIAM

A tribute to Robert L. “Gil” Gilbertson (1925-2011)



Our esteemed and beloved colleague, Robert L. “Gil” Gilbertson, passed away in late October, 2011. Gil was born on January 15, 1925 in Hamilton, MT to George and Eula Norris Gilbertson, grew up in Missoula, graduated from Missoula County High School in 1942, and on his 18th birthday joined the U.S. army, serving for three years. As a combat infantryman, Gil was awarded the Purple Heart, Bronze Star, and European Theater ribbon with two battle stars for his participation at the battle of Hürtgen Forest. This long battle killed and incapacitated over 33,000 men of the U.S. 1st Army. After discharge Gil studied forestry at University of Montana under the G.I. Bill of Rights, graduating with honors in 1949. In 1948 he married Patricia Park. The couple pursued their respective graduate programs at University of Washington. Upon Gil's completion of a MS in botany, they moved to Syracuse, N.Y. where he earned a Ph.D. in mycology and forest pathology at the State University of New York College of Forestry in 1954. Gil held successive faculty positions at University of Idaho (five years), State University of New York College of Forestry (eight years) and in 1967 settled at University of Arizona in the College of Agriculture, Department

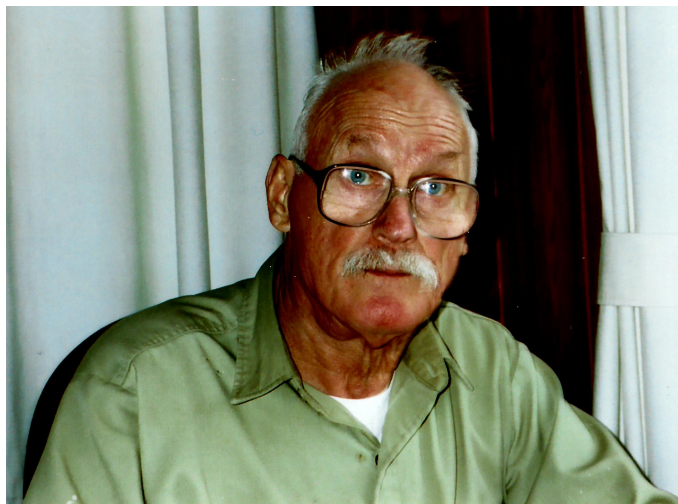
of Plant Pathology. He spent the next 30+ years teaching mycology and forest pathology, and studying the wood-rotting fungi of North America. Gil trained many students at the master's and doctorate level, and continued to mentor many into their own professional careers. His collaboration with the Norwegian scientist Leif Ryvarden resulted in publication of "The North American Polypores" and "The European Polypores", the most complete references for these groups to date. He continued his research and consulting after retiring in 1997. His son, Park, daughter, Joan, five grandchildren and wife, Celia Balfour, survive him.

Gil attended many WIFDWCs, and hosted the meeting in Tucson in September 1978. Gil was also very involved with the Mycological Society of America, serving as president in 1979. Recent past president Dave Hibbett states: “Gil’s work remains highly relevant today. For example, in 2011 Gil’s classic 1980 paper *Wood Rotting Fungi of North America* was cited in studies on fungal taxonomy, evolution, ecology, physiology, genomics, proteomics, and climate change biology. For a paper to be cited in such a wide range of articles more than thirty years after its publication is truly remarkable and it reflects the broad impact of Gil’s work.”

MaryLou Fairweather

IN MEMORIAM

Thomas H. (Tom) Laurent (1924-2012)



Thomas H. (Tom) Laurent, age 88 died peacefully at his home in Douglas on January 20, 2012. He was born January 7, 1924 in Clayton Georgia to Katherine Duggan Laurent and John Creighton Laurent. After graduating from the Riverside Military academy in 1942 he started college at the University of Idaho. His studies were interrupted by World War II. In 1943 he joined the United States Marine Corps. The majority of his enlistment was served as a radar operator in the Pacific Theater. He participated in the landings on and operations from the Islands of Kwajalein and Okinawa as well as the occupation of Japan at the end of the war. After his discharge he returned to the University of Idaho to complete his education. Tom worked summers as a mule

skinner supplying tree-lines and lookout stations in northern Idaho along the Salmon River.

After graduating from the University of Idaho with a degree in Forestry Tom went to Quincy, CA to begin his extensive career with the U.S. Forest Service. From Quincy he transferred to Pagosa Springs, Colorado where he trained and worked as a Snow Ranger. It was also where he met his true-love, Helen. When he was offered a Job at the Forestry Sciences Laboratory in Juneau, he asked her to come with him. They moved to Douglas in 1956 and were married on the 4th of July in the Douglas Community United Methodist Church. Aside from a couple of years in the early 1960s, when Tom went back to school to earn his master's degree in Forest Pathology at the University of Montana, their home was always in Douglas.

Tom was a great service to the communities of Douglas and Juneau as well as the entire state in many capacities. His research and study of the forests of the entire state has provided valuable information for their management. He was routinely called upon for avalanche control work and advice around Juneau and at the ski area located in the vicinity of the Dan Molar cabin. He was also assisted with the location and layout of the Eaglecrest Ski Area when it was originally established. He was a long time member of the National Ski Patrol both at Third Cabin and Eaglecrest. Tom also served a stint on the Douglas city planning commission prior to its annexation by Juneau.

Tom retired from the Forest Service in 1985. He spent his time building a cabin on the Taku River, hunting, fishing, bowling and working on various carpentry projects around his house in Douglas.

He was preceded in death by his wife Helen in 2010, and is survived by his sister Caroline McDaniel; three sons Tom Jr., Creighton, and Jim Laurent; their spouses Margaret Reddy, Martha Nariño-Torres, and Eleanor Laurent; and grand-children Lisa Dewitt-Nariño, Krystle Dewitt-Bean, Ivan Urrutia-Nariño and Abigail Laurent.

A memorial service will be held at 6pm Friday May 18th, 2012 at the Douglas Community United Methodist Church.



Those who spent time with Tom in the woods will remember a poem that he would recite as we hiked along. It was written by his long-term friend and colleague, ecologist Al Harris.

The deep, dark forest.

By A.S. Harris

Crunch, crunch, crunch,
Listen to 'em munch,
It's always time for lunch,
In the deep, dark forest.

The lepidopterees
Are eating up the trees
Until the winter freeze
In the deep, dark forest.

The parasites hover
And lunch upon each other
Including their own mother
In the deep, dark forest.

It's awful to behold
The creeping, crawling mold
Entwining in its fold
The deep, dark forest.

Oh Lord, deliver me
From these awful mysteries
As I crawl from tree to tree
In the deep, dark forest.

Paul Hennon

WIFDWC MEMBERS Active Members

<p>Judy Adams USDA FS-FHTE 2150 Centre Ave, Bld A, Suite 331 Fort Collins, CO 80526 970-295-5846 jadams04@fs.fed.us Last attended: 2011</p>	<p>Fred Baker USU - Dept of Wildland Resources 5230 Old Main Hill Logan, UT 84322-5230 435-797-2550 fred.baker@usu.edu Last attended: 2012</p>	<p>Peter Blenis Dept of Renewable Resources - University of Alberta Edmonton, Alberta T6G 2H1 780-492-0106 peter.blenis@ualberta.ca Last attended: 1995</p>	<p>John Browning Weyerhaeuser Forestry Research 505 N Pearl St, PO Box 420 Centralia, WA 98531 360-330-1721 john.browning@weyerhaeuser.com Last attended: 2012</p>
<p>Michelle Agne Oregon State University 1440 NW 15th St. Corvallis, OR 97331 206-384-9804 Michelle.Agne@oregonstate.edu Last attended: 2012</p>	<p>Tara Barrett PNW Research Station, USFS 1133 N. Western Ave Wenatchee, WA 98801 509-664-1715 tbarrett@fs.fed.us Last attended: 2012</p>	<p>James T. Blodgett Forest Health Management 8221 S Highway 16 Rapid City, SD 57702 605-716-2783 jblodgett@fs.fed.us Last attended: 2010</p>	<p>Beverly Bulao USDA Stanislaus National Forest 19777 Greenley Road Sonora, CA 95370 209-532-3681 X323 bbulao@fs.fed.us Last attended: 2012</p>
<p>Janice Alexander 290 Maynard St. San Francisco, CA 94112 415-337-7992 janice@cofod.net Last attended: 2010</p>	<p>Stephanie Beauseigle UBC- Forest Sciences 2424 Main Mall Vancouver, BC V6T 1Z4 604-822-8876 steph_beauseigle@hotmail.com Last attended: 2010</p>	<p>Kennedy Boateng UNBC 3333 University Way Prince George, BC V2N 4Z9 250-960-6659 boateng@unbc.ca Last attended: 2010</p>	<p>Lindsay Bulman Scion 49 Sala St Rotorua, New Zealand 3010 +64 7 343 5899 lindsay_bulman@scionresearch.com Last attended: 2006</p>
<p>Dionicio Alvarado El Colegio Posgrado Texcoco, Mexico dionicioyganoderma@gmail.com Last attended: 2012</p>	<p>Elisa Becker Canadian Forest Service-Pacific Forestry Center 506 West Burnside Road Victoria, BC V8Z 1M5 250-298-2382 Elisa.Becker@NRCan-RNCan.gc.ca Last attended: 2012</p>	<p>Simren Brar 1702 Nanaimo Street New Westminster, BC V3M 2G6 604-607-4758 simrenb@interchange.ubc.ca Last attended: 2011</p>	<p>Kelly Burns 740 Simms Street Golden, CO 80401 303-236-8006 ksburns@fs.fed.us Last attended: 2009</p>
<p>Peter Angwin USDA Forest Service 3644 Avtech Parkway Redding, CA 96002 530-226-2436 pangwin@fs.fed.us Last attended: 2012</p>	<p>Maia Beh UC Cooperative Extension-Humboldt County 5630 S. Broadway Eureka, CA 95503 530-771-7524 mmbeh@ucdavis.edu Last attended: 2012</p>	<p>Anna Brown Forest Research Alice Holt Lodge Farnham, Surrey GU10 4LH UK 4.4782787365e+011 anna.brown@forestry.gsi.gov.uk Last attended: 2006</p>	<p>Roger Burnside State of Alaska, DNR Division of Forestry 550 West 7th Avenue, Suite 1450 Anchorage, AK 99501- 907-229-5754 roger.burnside@alaska.gov</p>

Kim Camilli
Cal Fire/Cal Poly
P.O. Box 1054
Santa Margarita, CA 93453
512-497-3687
kim.camilli@fire.ca.gov
Last attended: 2012

Phil Cannon
USDA FS, 1323 Club Dr.
Vallejo, CA 94592
707-562-8913
pcannon@fs.fed.us
Last attended: 2012

Ann Marie Casper
527 E. Laurel St.
Fort Collins, CO 80524
845-242-5759 0
Last attended: 2010

Kristen L. Chadwick
USFS FHP
16400 Champion Way
Sandy, OR 97055
503-668-1474
kchadwick@fs.fed.us
Last attended: 2012

Gary Chastagner
Washington State University, Res. &
Ext. Center
2606 West Pioneer
Puyallup, WA 98371
253-445-4528
chastag@wsu.edu
Last attended: 2011

Michelle Cleary
Swedish Agricultural University
750 07 Uppsala, Sweden
018-672794
michelle.cleary@slu.se
Last attended: 2011

Christy Cleaver
Colorado State University
Fort Collins, CO 80523
303-907-8718
ccleaver@rams.colostate.edu
Last attended: 2012

David Conklin
333 Broadway SE
Albuquerque, NM 87102
505-842-3288
david.conklin@lifeboatearth.org
Last attended: 2009

Mike Cruickshank
Canadian Forest Service
506 West Burnside Road
Victoria, BC V8Z 1M5
250-363-0641
mcruiicks@nrcan.gc.ca
Last attended: 2012

Robert Cruz
324 25th Street
Ogden, UT 84401
801-625-5162
rcruz@fs.fed.us
Last attended: 2008

Tom DeGomez
NAU
PO Box 15018
Flagstaff, AZ 86001
928-523-8385
degomez@ag.arizona.edu
Last attended: 2007

Annette Delfino-Mix
2480 Carson Rd.
Placerville, CA 95667
530-295-3023
amix@fs.fed.us
Last attended: 2007

Gregg DeNitto
USDA Forest Service, FHP
P.O. Box 7669
Missoula, MT 59807
406-329-3637
gdenitto@fs.fed.us
Last attended: 2010

Marla Downing
2150 Center Ave, Bldg A, Suite 311
Fort Collins, CO 80526
970-295-5843
mdowning@fs.fed.us
Last attended: 2007

Megan Dudley
Colorado State University
1636 W. Stuart St.
Fort Collins, CO 80526
970-222-9558
meg.dudley@rams.colostate.edu
Last attended: 2012

Nick Dudley
Hawaii Ag Research Center
Hawaii
808-391-5421
ndudley@harc-hspa.com
Last attended: 2012

Ruben D. Elias-Román
El Colegio de Postgraduados
Texcoco, Mexico 56230
edamian@colpos.mx
Last attended: 2012

Marianne Elliott
WSU - Puyallup Research and
Extension Center
2606 West Pioneer
Puyallup, WA 98371
253-445-4596
melliott2@wsu.edu
Last attended: 2011

MaryLou Fairweather
USDA Forest Service
2500 South Pine Knoll Drive
Flagstaff, AZ 86001
928-556-2075
mfairweather@fs.fed.us
Last attended: 2012

Lina Farfan
University of British Columbia Dept of
Forest Sciences
Vancouver, BC V6T 1Z4
778-928-4346
linafarfan@hotmail.com
Last attended: 2011

Brennan Ferguson
Ferguson Forest Pathology Consulting
P.O. Box 2127
Missoula, MT 59806-2127
406-239-7761
brennan@fergusonforestpathology.com
Last attended: 2008

Gregory Filip
USDA Forest Service - FHP
PO Box 3623
Portland, OR 97208-3623
503-808-2997
gmfilip@fs.fed.us
Last attended: 2012

Susan Frankel
USDA FS, PSW Res, Station
800 Buchanan Street
Albany, CA 94708
510-559-6472
sfrankel@fs.fed.us
Last attended: 2012

Martin Frye
 UC Davis - Tahoe Env. Research Ctr
 291 Country Club Dr., 3rd Floor TCES
 Incline Village, NV 89451
 530-546-3014
 martinjfrye@gmail.com
 Last attended: 2012

Amy Gannon
 Montana DNRC
 2705 Spurgin Road
 Missoula, MT 59804
 406-542-4283
 agannon@mt.gov
 Last attended: 2012

Matteo Garbelotto
 UC Berkeley
 54 Mulford Hall
 Berkeley, CA 94720
 510-643-4282
 matteog@berkeley.edu
 Last attended: 2012

Nancy Gillette
 2525 Hill Court
 Berkeley, CA 94708
 510-559-6474
 ngillette@fs.fed.us
 Last attended: 2010

Jessie Glaeser
 USDA Forest Service
 One Gifford Pinchot Dr.
 Madison, WI 53726
 608-231-9215
 jglaeser@fs.fed.us
 Last attended: 2012

Ellen Michaels Goheen
 USDA Forest Service - FHP
 2606 Old Stage Road
 Central Point, OR 97502
 541-858-6126
 egoheen@fs.fed.us
 Last attended: 2012

Scott Golden
 Boulder County POS
 5201 Saint Vrain Road
 Longmont, CO 80503
 303-678-6209
 sgolden@bouldercounty.org
 Last attended: 2009

Betsy Goodrich
 USDA FS
 240 West Prospect Road
 Fort Collins, CO 80526
 970-498-1029
 bgoodrich@fs.fed.us
 Last attended: 2012

Tom Gordon
 UC Davis, Department of Plant
 Pathology
 One Shields Ave
 Davis, CA 95616
 530-754-9893
 trgordon@ucdavis.edu
 Last attended: 2012

Laura Gray
 Centre for Forest Biology,
 University of Victoria
 PO Box 3020, STN CSC
 Victoria, BC V8W 3N5
 250-857-0966
 lkgray@unvic.ca
 Last attended: 2012

Nancy Grulke
 USDA, FS, WWETAC
 3160 NE 3rd Street
 Prineville, OR 97754
 541-416-6583
 ngrulke@fs.fed.us
 Last attended: 2012

John C. Guyon II
 USDA Forest Service, FHP
 4746 S. 1900 E.
 Ogden, UT 84403
 801-476-9720 ext 218
 jguyon@fs.fed.us
 Last attended: 2007

John-Erich Haight
 USDA-FS Northern Research Station
 One Gifford Pinchot Drive
 Madison, WI 53726
 608-231-9571
 jhaight@fs.fed.us
 Last attended: 2010

John Hanna
 USDA Forest Service
 1221 South Main St.
 Moscow, ID 83843
 208-883-2337
 jhanna@fs.fed.us
 Last attended: 2011

Chris Hansen
 UNBC 3333 University Way
 Prince George, BC V2N 4Z9
 250-960-6659
 chansen@unbc.ca
 Last attended: 2010

Jeri Lyn Harris
 USDA Forest Service
 470 Simms St.
 Golden, CO 80401
 303-275-5155
 jharris@fs.fed.us
 Last attended: 2005

Linda Haugen
 US Forest Service
 1992 Folwell Avenue
 St. Paul, MN 55108
 651-649-5029
 lhaugen@fs.fed.us

Erin Havard
 B.C. Ministry of Forests & Range
 Bag 6000, 3333 Tatlow Rd
 Smithers, BC V0J 2N0
 250-847-6388
 erin.i.havard@gov.bc.ca
 Last attended: 2010

Von Helmuth
 US Forest Service - FHP
 3815 Schreiber Way
 Coeur d'Alene, ID 83815
 208.765.7342
 vhelmuth@fs.fed.us
 Last attended: 2010

Paul Hennon
 USDA Forest Service, Forestry
 Sciences Lab, 11305 Glacier Highway
 Juneau, AK 99801
 907-586-8769
 phennon@fs.fed.us
 Last attended: 2012

Robert Hodgkinson
 B.C. Ministry of Forests & Range
 1011 - 4th Ave.
 Prince George, BC V2L 3H9
 250-565-6122
 Robert.Hodgkinson@gov.bc.ca

Ted (E.H.) Hogg
 Northern Forestry Centre, CFS-NRCan
 5320-122 Street
 Edmonton, AB T6H 3S5
 780-435-7225
 ted.hogg@nrcan.gc.ca
 Last attended: 2007

Brian Howell
 740 Simms St.
 Golden, CO 80401
 303-236-8001
 behowell@fs.fed.us
 Last attended: 2007

Abbey Hudler
Utah State University
5230 Old Main Hill
Logan, UT 84322-5230
435-513-9160
abbeyhudler@yahoo.com
Last attended: 2012

Joe Hulbert
Oregon State University
PO Box 236
Corvallis, OR 97339
541-908-5129
hulbertj@science.oregonstate.edu
Last attended: 2012

Karen Hutten
University of Washington
PO Box 352100
Seattle, WA 98195
360-460-5718
huttenk@u.washington.edu
Last attended: 2012

Louis Iverson
USFS No. Research Station
359 Main Road
Delaware, OH 43015
740-368-0097
liverson@fs.fed.us
Last attended: 2012

Marcus Jackson
USDA Forest Service, FHP
200 E. Broadway
Missoula, MT 59807
406-329-3282
mbjackson@fs.fed.us
Last attended: 2012

William Jacobi
Colorado State University,
Dept of BSPPS
129 Plant Science Building, 1177
Campus Delivery
Fort Collins, CO 80523-1177
970-491-6927
william.jacobi@colostate.edu
Last attended: 2012

James Jacobs
625 Robert St. N
St. Paul, MN 55155
612-799-4384
jacobs.jamesj@gmail.com

Chamile Jensen
UC Davis - Tahoe Environmental
Research Ctr
291 Country Club Dr., 3rd Floor TCES
Incline Village, NV 89451
530-546-3014
pemaloney@ucdavis.edu
Last attended: 2012

Nathan Johnson
University of Washington
PO Box 352100
Seattle, WA 98195
781-927-5341
ngjohnso@gmail.com
Last attended: 2011

Randy Johnson
USDA Forest Service, Research and
Development
1601 North Kent Street, Rosslyn Plaza
C 4th Floor
Arlington, VA 22209
703-605-5178
randyjohnson@fs.fed.us
Last attended: 2012

Tyler Jones
Hawaii Agriculture Research
PO Box 100
Kunia, HI 96759
808-927-7508
tylerjones@gmail.com
Last attended: 2012

William Jones
USDA Forest Service, Southern
Research Station
200 Weaver Blvd
Asheville, NC 28801
828-259-0526
wejones@fs.fed.us
Last attended: 2011

Alan Kanaskie
Oregon Department of Forestry
2600 State Street
Salem, OR 97310
503-945-7397
akanaskie@odf.state.or.us
Last attended: 2011

Cynthia Kanner
MT - DNRC
PO Box 489
Darby, MT 59829
cynkanner@gmail.com
Last attended: 2011

Holly Kearns
USDA Forest Service
16400 Champion Way
Sandy, OR 97055
503-668-1475
hkearns@fs.fed.us
Last attended: 2012

Mahsa Khorasani
University of Washington School of
Envir and For Sciences
PO Box 352100
Seattle, WA 98195
425-269-7829
mkh2612@uw.edu
Last attended: 2012

Ned Klopfenstein
USDA Forest Service, RMRS
1221 S. Main St.
Moscow, ID 83843
208-883-2310
nklopfenstein@fs.fed.us
Last attended: 2012

Jennifer Kluttsch
240 W. Prospect
Fort Collins, CO 80526
970-498-1387
jkluttsch@gmail.com
Last attended: 2009

Chris Konchalski
UNBC 3333 University Way
Prince George, BC V2N 4Z9
250-964-9314
konchal@unbc.ca
Last attended: 2010

Harry H. Kope
BC Ministry of Forests, Lands and
Natural Resource Operations
9th floor, 727 Fisgard Street
Victoria, BC V9B 1K7
250-387-5225
harry.kope@gov.bc.ca
Last attended: 2010

Anna Leon
Washington State University
2606 W. Pioneer
Puyallup, WA 98371
253-820-7455
anna.leon100@email.wsu.edu
Last attended: 2012

Kathy Lewis
UNBC
3333 University Way
Prince George, BC V2N 4Z9
250-960-6659
lewis@unbc.ca
Last attended: 2011

Will Littke
Weyerhaeuser Forestry
32901 Weyerhaeuser Way S
Federal Way, WA 98001
253-924-6995
will.littke@weyerhaeuser.com
Last attended: 2012

Blakey Lockman
USDA Forest Service, FHP
200 E. Broadway
Missoula, MT 59807
406-329-3189
blockman@fs.fed.us
Last attended: 2012

Daniel Lux
Alberta Sustainable Resource
Development
7th Floor Great West Life Bldg.
9920-108 Street
Edmonton, AB T5K 2M4
780-644-2246
daniel.lux@gov.ab.ca

Martin MacKenzie
USDA Stanislaus National Forest
1977 Greenley Rd
Sonora, CA 95370
209 532 3671 ext 242
mmackenzie@fs.fed.us
Last attended: 2012

Tom Maertens
University of British Columbia Dept of
Forest Sciences
Vancouver, BC V6T 1Z4
604-710-3410
maertens@forestmail.com
Last attended: 2011

Helen Maffei
USFS Central Oregon FHP Service
Center
63095 Deschutes Market Rd.
Bend, OR 97701
541-383-5591
hmmaffei@fs.fed.us
Last attended: 2012

Patricia Maloney
UC Davis, Tahoe Environmental
Research Ctr
291 Country Club Dr., 3rd Floor TCES
Incline Village, CA 89451
530-546-3014
pemaloney@ucdavis.edu
Last attended: 2012

Danielle Martin
USDA Forest Service
180 Canfield Street
Morgantown, WV 26505
304-285-1531
dkmartin@fs.fed.us
Last attended: 2011

Robert Mathiasen
Northern Arizona University
PO Box 15018
Flagstaff, AZ 86001
928-523-0882
robert.mathiasen@nau.edu
Last attended: 2011

Kathleen McKeever
1614 4th Ave NW, Apt. A
Puyallup, WA 98371
845-750-7461
kmmckeey@wsu.edu
Last attended: 2010

Michael McWilliams
Oregon Department of Forestry
2600 State Street
Salem, OR 97310
503-945-7396
mmcwilliams@odf.state.or.us
Last attended: 2012

Rosario Medel
University in Xalapa
Xalapa, Mexico
medel.rosario@gmail.com
Last attended: 2012

Heather Mehl
UC Davis
615 East 8th St., Apartment B
Davis, CA 95616
530-320-5475
hkmehl@ucdavis.edu
Last attended: 2012

Maria Mircheva
Sugar Pine Foundation
1458 Mt Raimier Dr South
Lake Tahoe, CA 96150
650-814-9565
maria.mircheva@gmail.com
Last attended: 2012

Bruce Moltzan
USDA Forest Health Protection
1601 N. Kent St. RPC7-FHP
Arlington, VA 22209
703-605-5344
bmoltzan@fs.fed.us
Last attended: 2012

Jeff Moore
USDA Forest Service R-5
jwmoore@fs.fed.us
Last attended: 2012

Leif Mortenson
Oregon State University
204 Peavy Hall
Corvallis, OR 97331
503-901-5666
singletrack82@yahoo.com
Last attended: 2012

Robin Mulvey
USDA Forest Service
11305 Glacier Highway
Juneau, AK 99801
907-586-7971
rlmulvey@fs.fed.us
Last attended: 2012

Isabel Munck
1045 Litch Ct.
Reno, NV 89509
775-750-6779
imunck@cabnr.unr.edu
Last attended: 2008

Michael Murray
BC Ministry of Forests, Lands and
Natural Resource Operations
#401 - 333 Victoria St.,
Nelson, BC V1L 4K3
250-825-1173
michael.murray@gov.bc.ca
Last attended: 2012

Sarah Navarro
Oregon State University
2082 Cordley Hall
Corvallis, OR 97331
408-644-0508
navarros@science.oregonstate.edu
Last attended: 2012

Ron Neilson
3200 SW Jefferson Way
Corvallis, OR 97333
541-750-7303
neilson@fsl.orst.edu

Danny Norlander
New Mexico State Forestry Division
1220 South St. Francis Drive
Santa Fe, NM 87505
541-829-0046
daniel.norlander@state.nm.us
Last attended: 2011

Steve Oak
USDA Forest Service, Southern
Region FHP
200 W.T. Weaver Blvd
Asheville, NC 28804
828-257-4322
soak@fs.fed.us
Last attended: 2008

Brent Oblinger
USDA Forest Service
1731 Research Park Drive
Davis, CA 95618
530-902-7134
boblinger@fs.fed.us
Last attended: 2012

Joeseph O'Brien
USDA Forest Service
1992 Folwell Ave
St. Paul, MN 55108
651-649-5266
jobrien@fs.fed.us

Eunsung Oh
Dep. of Forest Insect Pests and
Diseases, Korea Forest Research
Institute
Seoul, South Korea 130-782
82-2-961-2669
esoh75@hotmail.com
Last attended: 2008

Forrest L. Oliveria
USDA Forest Service - FHP
2500 Shreveport Highway
Pineville, LA 71360
318-473-7294
foliveria@fs.fed.us
Last attended: 2011

Daniel Omdal
WA Department of Natural Resources
1111 Washington St. SE
Olympia, WA 98504
360-902-1692
dan.omdal@dnr.wa.gov
Last attended: 2012

Nancy Osterbauer
Oregon Dept. of Agriculture
635 Capitol St. NE
Salem, OR 97301
503-986-4620
nosterbauer@oda.state.or.us

Donald R. Owen
California Dept. of Forestry and Fire
Protection
6105 Airport Rd.
Redding, CA 96002
530-224-2494
don.owen@fire.ca.gov
Last attended: 2012

Tim Owen
UNBC
3333 University Way
Prince George, BC V2N 4Z9
250-617-0875
owen@unbc.ca
Last attended: 2011

Jennifer Parke
Oregon State University Dept. of Crop
and Soil Science
Corvallis, OR 97331
541-737-8170
Jennifer.Parke@oregonstate.edu

Catherine Parks
USDA Forest Service
1401 Gekeler Lane
LaGrande, OR 97850
541-962-6531
cparks01@fs.fed.us

Ebba Peterson
Oregon State University
280 Peavy Hall
Corvallis, OR 97331
541-737-5242
petersoe@science.oregonstate.edu
Last attended: 2012

Pamela Phillips
18813 SE Lake Holm Road
Auburn, WA 98092
253-217-8447
pafphillips@comcast.net
Last attended: 2011

Rebecca Powell
Colorado State University
129 Plant Science Building,
1177 Campus Delivery
Fort Collins, CO 80523
720-480-8214
rebeccashifler@yahoo.com
Last attended: 2011

Jay Pscheidt
Oregon State University
2082 Cordley Hall
Corvallis, OR 97331
pscheidt@science.oregonstate.edu
Last attended: 2011

Amy Ramsey-Kroll
WA Dept. of Natural Resources
PO Box 47037, 1111 Washington St. SE
Olympia, WA 98504
360-902-1309
amy.kroll@dnr.wa.gov
Last attended: 2011

Tod Ramsfield
Northern Forestry Centre
Private Bag 3020
Edmonton, Alberta
780-435-7394
Tod.Ramsfield@nrcan.gc.ca
Last attended: 2011

Richard Reich
BC Ministry of Forests, Lands and
Natural Resource Operations
1044 5th Ave, 5th Floor
Prince George, BC V2L 5G4
250-565-6203
richard.reich@gov.bc.ca
Last attended: 2012

Kathy Riley
WSU Research and Extension Center
2606 West Pioneer
Puyallup, WA 98371
253-445-4625
ktriley@wsu.edu

Karen Ripley
DNR, Resource Protection Division
PO Box 47037
Olympia, WA 98504-7037
360-902-1691
karen.ripley@dnr.wa.gov

Gabriela Ritokova
Dept of Forest Engineering, Resources
and Management,
Oregon State University
027 Peavy Hall
Corvallis, OR
415-640-9663
gabriela.ritokova@oregonstate.edu
Last attended: 2012

David Rizzo
University of California - Davis
Department of Plant Pathology
Davis, CA 95616
530-754-9255
dmrizzo@ucdavis.edu
Last attended: 2003

Jonas Ronnberg
Southern Swedish Forest Research
SE-23053 Alnarp, Sweden
0706-727 643
jonas.ronnberg@ess.slu.se
Last attended: 2010

Amy Ross-Davis
USDA Forest Service
1221 South Main Street
Moscow, ID 83843
208-883-2337
arossdavis@fs.fed.us
Last attended: 2012

David Rusch
BC Ministry of Forests, Lands and
Natural Resource Operations
1959 S. Lakeside Dr.
Williams Lake, BC V2G 5G1
250-392-4488
david.rusch@gov.bc.ca
Last attended: 2012

Dave Russell
481 Penny Lane
Grants Pass, OR 97527
541-479-3446
dr1855@q.com
Last attended: 2009

Daniel Ryerson
USDA Forest Service, Forestry and
Forest Health
333 Broadway Blvd., SE
Albuquerque, NM 87102
505-842-3285
dryerson@fs.fed.us

Angel Saavedra
USDA Forest Service
1133 N Western Avenue
Wenatchee, WA 98801
509-664-9223
alsaavedra@fs.fed.us
Last attended: 2012

Hugh Safford
USDA Forest Service
1323 Club Dr.
Vallejo, CA 94592
530-219-0898
hughsafford@fs.fed.us
Last attended: 2012

Denis Sandanov
Buryat State University in Ulan Ude
1458 Mt Raimier Dr ,
707-365-3726
denis.sandanov@gmail.com
Last attended: 2012

Bill Schapp
USDA Forest Service
8221 South Highway 16
Rapid City, SD 57702
605-716-2797
bschapp@fs.fed.us

Bob Schlub
University of Guam, ANR / CES /
CNAS University Station
Mangilao, Guam 96923
671-735-2089
rslclub@uguam.uog.edu
Last attended: 2012

Anna Schoettle
USDA Forest Service, Rocky
Mountain Research Station
240 WEST PROSPECT RD
Fort Collins, CO 80526
970-498-1333
aschoettle@fs.fed.us
Last attended: 2009

Mark Schultz
USDA Forest Service
11305 Glacier Hwy
Juneau, AK 99801-8628
907-586-8883
Mschultz01@fs.fed.us

Simon Shamoun
NRC - Pacific Forestry Centre
506 West Burnside Road
Victoria, BC V8Z 1M5
250-298-2358
sshamoun@nrcan.gc.ca
Last attended: 2011

David C. Shaw
Oregon State University
204 Peavy Hall
Corvallis, OR 97331
541-737-2845
dave.shaw@oregonstate.edu
Last attended: 2012

Michael Simpson
USFS FHP Central Oregon Service
Center
63095 Deschutes Market Rd.
Bend, OR 97701
541-383-5575
mlsimpson@fs.fed.us
Last attended: 2012

Laura Sims
Oregon State University
2074a Cordley Hall
Corvallis, OR 97331
541-737-5242
simslla@science.oregonstate.edu
Last attended: 2012

Eric L. Smith
USDA Forest Service - FHTE
2150 Centre Avenue, Building A, Ste 331
Ft Collins, CO 80526-8121
970-295-5841
elsmith@fs.fed.us
Last attended: 2012

Tom Smith
California Dept. of Forestry and Fire
Protection
573 40th Street
Sacramento, CA 95819
916-599-6882
tom.smith@fire.ca.gov
Last attended: 2012

Richard Sniezko
USDA Forest Service
Dorena Genetic Resource Center
34963 Shoreview Road
Cottage Grove, OR 97424
541-767-5716
rsniezko@fs.fed.us
Last attended: 2012

Keith Sprengel
USDA Forest Service
16400 Champion Way
Sandy, OR 97055
503-668-1476
ksprengel@fs.fed.us

Glen R. Stanosz
Dept. Plant Pathology, Univ.
Wisconsin-Madison
1630 Linden Drive
Madison, WI 53706
608-265-2863
grs@plantpath.wisc.edu
Last attended: 2008

Jane Stewart
University of Idaho
Moscow, ID 83843
509-338-5038
jestewart@uidaho.edu
Last attended: 2011

Jeff Stone
Dept of Botany and Plant Pathology
Oregon State University
Cordley 2082
Corvallis, OR 97331-2902
541-737-5260
stonej@science.oregonstate.edu

Norma Stromberg-Jones
Box 40
McBride, BC V0J 2E0
250-569-3788
Norma.StrombergJones@gov.bc.ca
Last attended: 2010

Rona Sturrock
NRC - Pacific Forestry Centre
506 West Burnside Road
Victoria, BC V8Z 1M5
250-298-2376
Rona.Sturrock@nrcan.gc.ca
Last attended: 2011

Kat Sweeney
OSU
2082 Cordley Hall
Corvallis, OR 97331-2902
541-737-3573
sweeneka@science.oregonstate.edu
Last attended: 2012

Cassandra Swett
UC Davis
One Shields Ave
Davis, CA 95616
530-754-7634
clswett@ucdavis.edu
Last attended: 2012

Borys Tkacz
USDA Forest Service - FHP
1601 N. Kent Street
Arlington, VA 22209
703-605-5343
btkacz@fs.fed.us
Last attended: 2011

Yana Valachovic
UC Cooperative Extension
5630 S. Broadway
Eureka, CA 95503
707-445-7351
yvala@ucdavis.edu
Last attended: 2012

Detlev Vogler
USDA, FS, PSW Station, Institute of
Forest Genetics
2480 Carson Road
Placerville, CA 95667-5107
530-621-6881
dvogler@fs.fed.us
Last attended: 2012

Jim Walla
NDSU Plant Pathology Dept 7660,
PO Box 6050
Fargo, ND 58208
701-231-7069
j.walla@ndsu.edu
Last attended: 2011

Beth Willhite
Mt. Hood National Forest
16400 Champion Way
Sandy, OR 97055
503-668-1477
bwillhite@fs.fed.us

Lori Winton
USDA Forest Service
3301 C St. Suite 202
Anchorage, AK 95547
907-743-9460
lmwinton@fs.fed.us
Last attended: 2010

Bill Woodruff
USDA Forest Service
2550 Riverside Drive
Susanville, CA 96130
530-252-6680
wwoodruff@fs.fed.us
Last attended: 2012

Alex Woods
BC Ministry of Forests, Lands and
Natural Resource Operations
Bag 6000
Smithers, BC V0J 2N0
250-847-6382
Alex.Woods@gov.bc.ca
Last attended: 2012

Jim Worrall
Forest Service
216 N. Colorado St.
Gunnison, CO 81230
970-642-1166
jworrall@fs.fed.us
Last attended: 2009

Jessica Wright
USDA Forest Service
1731 Research Park Drive
Davis, CA 95618
530-759-1742
jessicawright@fs.fed.us
Last attended: 2012

Yun Wu
USDA Forest Service
180 Canfield Street
Morgantown, WV 26505
304-285-1594 ywu@fs.fed.us
Last attended: 2012

Doug Wulff
USDA Forest Service
3815 Schreiber Way
Coeur d'Alene, ID 83815
208-765-7344
dwulff@fs.fed.us

Paul Zambino
USDA Forest Service
3815 Schreiber Way
Coeur d'Alene, ID 83815
208-765-7201
pzambino@fs.fed.us
Last attended: 2012

Stefan Zeglen
BC Ministry of Forests, Lands and
Natural Resource Operations
2100 Labieux Road
Nanaimo, BC V9T 6E9
250-751-7108
stefan.zeglen@gov.bc.ca
Last attended: 2012

WIFDWC MEMBERS

Honorary Live Members

Jerry Beatty
6028 SE Reed College Place
Portland, OR 97202
503-810-8723
jeromebeatty@gmail.com

Clive Brasier
Forest Research Agency
Alice Holt Lodge
Farnham Surrey, UK GU10 4LH

Jim Byler
1523 West Woodland Drive
Dalton Gardens, ID 83815
208-972-7442
jjbyler@aol.com

Sally Campbell
6028 SE Reed College Place
Portland, OR 97202
503-810-8717
campbellsally@comcast.net

Charles Driver
2019 Edith Ave
Enumclaw, WA 98022
360-802-3083

Robert L. Edmonds
4517 48th Ave NE
Seattle, WA 98105
206-523-6913
bobe@u.washington.edu

David Etheridge
3941 Oakdale Place
Victoria BC V8N 3B6

Mike Finnis
1888 Gonzales Ave.
Victoria, BC V8N 3B6

Brian Geils
2500 South Pine Knoll Drive
Flagstaff, AZ 86001
928-556-2076
bgeils@fs.fed.us

Linnea Gillman
3024 S Winona Ct.
Denver, CO 80236

James Ginns
1970 Sutherland Road
Penticton, BC V2A 8T8
250-492-9610
ginnsj@shaw.ca

Don Goheen
2564 Sterling Creek Road
Jacksonville, OR 97530
541 899-1449
edgoheen@jeffnet.org

Don Graham
5702 NE 88th Court
Vancouver, WA 98662
360-892-8811
dongram@pacifier.com

Jim Hadfield
1133 N. Western Ave.
Wenatchee, WA 98801
509-664-9215
jshadfield@fs.fed.us

Susan K. Hagle
379 Lee Road
Harper, ID 83552
208 926-4545
2lazyh@wildblue.net

Everett Hansen
OSU - Botany and Plant Pathology
2082 Cordley Hall
Corvallis, OR 97331
541-737-5243
hansene@science.oregonstate.edu

John H. Hart
1390 Curt Gowdy Drive
Cheyenne, WY 82009
huntwyoming@aol.com

Alan Harvey
4100 Wenview Court West
Richland, WA 99353
509-628-3124
asharvey100@msn.com

Diane Hildebrand
10408 W 83rd Drive
Arvada, CO 80005
360-903-2891
hildebranddiane@yahoo.com

Ray Hoff
907 East 7th Street
Moscow, ID 83483

James T. Hoffman
USDA Forest Service
1249 South Vinnell Way, Suite 200
Boise, ID 82046
208-373-4221
jthoffman@fs.fed.us

Richard Hunt
1844 Stamps Rd.
Duncan, BC V9L 5W1
250-746-7259
ribicola@gmail.com

Bob James
520 Se Columbia River Drive #116
Vancouver, WA 98661
360-936-5658
treejpathman6@yahoo.com

David Johnson
12851 W Asbury Place
Lakewood, CO 80288

John Kliejunas
5305 Lightwood Drive
Concord, CA 94521
925-682-4825
kliejunas@comcast.net

John Laut
4700 E. Main St., # 1529
Mesa, AZ 85025
480-620-3402
johnglaut@gmail.com

Leon Lemadeleine
PO Box 1130
Morgan, UT 84050
801-845-9173

Katy Mallams
USDA Forest Service
2606 Old Stage Road
Central Point, OR 97502
541-858-6124
July4_girl@yahoo.com

Otis Maloy
1036 Wallen Road
Moscow, ID 83843
208-883-0940
otismaloy@cpinternet.com

Walter Mark
14612 White Pine Way
La Pine, OR 97739
805-305-2553
wmark@calpoly.edu

Neil E. Martin
514 South Howard
Moscow, ID 83843
208-882-7049
jandnmart@moscow.com

Arthur H. McCain
1 Hilldale Road
Lafayette, CA 94549
925-284-9632
mccain@nature.berkeley.edu

Geral McDonald
553 Old Moscow Road
Pullman, WA 99163
509-332-0352
geral.mcdonald4@gmail.com

Alex Molnar
241 - 1700 Comox
Comox, BC V9M 4H4
250-890-2259

Duncan Morrison
1487 Stellys Cross Road
Saanichton, BC V8M 1S8
250 652 3281
armillaria@shaw.ca

John Muir
2881 Heath Drive
Victoria, BC V9A 2I6
250-477-1805
johnmuir@consultant.com

Earl Nelson
2175 Condor drive
Redmond, OR 97756
541-504-0685
bigearl35@aol.com

Thomas H. Nicholls
P.O. Box 63,
W7283 Walnut St.
FIFIELD, WI 54524
715 762-3076
nich002@umn.edu

Vidar Nordin
Box 2368, Stn. D
Ottawa, ON K1P 5W5
613-234-7478
vidar.nordin@gmail.com

Fred Peet
1210 Marin Park Drive
Brentwood Bay, BC V8M 1G7
250-652-1344
fpeet@eidetic.bc.ca

Roger S. Peterson
1750 Camino Corrales
Santa Fe, NM 87505
505-983-7559
RogPete@aol.com

Glenn Peterson
3817 Dudley
Lincoln, NE 68503
402-464-3696

John Pronos
21937 El Oso Way
Sonora, CA 95370
209-532-6221
johnpronos@gmail.com

Jerry W. Riffle
6086 E. George Street
Syracuse, IN 46567
574-457-3065
jerry.riffle@kconline.com

Kenelm Russell
8143 Evergreen Dr. NE
Olympia, WA 98506
360-943-8199
kenelmrussell@msn.com

Robert Scharpf
Quartz Hill Vineyard
8548 Mosquito Road
Placerville, CA 95667
530-622-8315
quartzhillvineyard@gmail.com

Mike Schomaker
5400 Vardon Way
Fort Collins, CO 80528-9114
970-223-1929
Michael.Schomaker@colostate.edu

John W. Schwandt
USDA Forest Service
3815 Schreiber Way
Coeur d'Alene, ID 83815
208-765-7415
jschwandt@fs.fed.us

Terry Shaw
3160 NE Third Street
Prineville, OR 97754
541-416-6618
charlesgshaw@fs.fed.us

Wayne Sinclair
420 Taylor Place
Ithaca, NY 14850
was1@cornell.edu

Michael Srago
7006 Potrero Ave
El Cerrito, CA 93540
510-232-7092
msrago@comcast.net

James Stewart
3028 Covington St.
Fairfax, VA 22031-2011

Jack Sutherland
1963 St. Ann
Victoria, BC V8R 5V9
JackSutherland@shaw.ca

Ag Tegethoff
11750 E. Sneller Vista Dr
Tucson, AZ 85749

Walt Thies
3317 NW Firwood Drive
Corvallis, OR 97330
541-752-5214
wgthies@comcast.net

Bart Van Der Kamp
Dept Forest Sciences, UBC
Vancouver, BC V6T 1Z4
604 946-4673
vdkamp@telus.net

Eugene VanArsdel
62 Lagarto
Tijeras, NM 87059
505-286-4116
epvan@live.com

Allen Van Sickle
4436 Rangemount Pl
Victoria, BC V8N SL6
250-721-0734

Gordon Wallis
4720 Spring Rd. RR#3
Victoria, BC V8X 3X1

Roy Whitney
47 Cumberland Dr. NW
Calgary, AB T2K 1S8
403-284-5650
drrroot@telus.net

Stuart Whitney
2206 Ayum Rd.
Sooke, BC V9Z 0E7
250-642-5546
stuwhitney@shaw.ca

Ralph Williams
9650 S Powerline Rd
Nampa, ID 83686-9408

We have lost track of the following Honorary Life Members. If you know of their whereabouts please contact Fred Baker at 435-797-2550 or fred.baker@usu.edu

Paul Aho
Ed Andrews
Alvin Funk
Bob Harvey
John Hopkins
Paul Lightle
Don Norris
Art Partridge

Jack Roff
Jim Trappe
Lori Trummer
Bob Tinnin
Mike Sharon
Richard B Smith
Ed Wood
Conrad Wessela

**WIFDWC MEMBERS
DECEASED MEMBERS**

Stuart "Stuie" Andrews
Jesse Bedwell
Robert Bega
Warren Benedict
John Bier
Richard "Dick" Bingham
Bill Bloomberg
Roy Bloomstrom
Thomas "Buck" Buchanan
Don Buckland
Hubert "Hart" Bynum
Elmer Canfield
Fields Cobb
Ross Davidson
Oscar Dooling
Norm Engelhart
Ray Foster
Dave French
Robert Gilbertson
Lake S. Gill
Clarence "Clancy" Gordon
John Gynn
John Hansbrough
Hans Hansen
Homer Hartman
George Harvey
Frank G. Hawksworth
Dwight Hester
Tommy Hinds
Brenton Howard
John Hunt
Paul Keener
James Kimmey
Andrea Koonce
Tom Laurent
Don Leaphart
Tom McGrath
Neil E. McGregor
Jim Mielke
D. Reed Miller
Vergil Moss
Harrold Offord
Nagy Oshima
Lee Paine
John Palmer
John "Dick" Parmeter Jr.
Clarence Quick
Lew Roth
Dave Shultz
Peter Schutt
Charles G. Shaw
Keith Shea
Albert Slipp
Willhelm Solheim
Albert Stage
Phil Thomas
Willis Wagener
Charles "Doc" Waters
Larry Weir
Ed Wicker
John Woo
Ernest Wright
Wolf Ziller

Group Photos WIFDWC 2012



Group 1

Back Row: Fred Baker, Eric Smith, Gregory Filip, Robin Mulvey
Middle Row: Laura Gray, Ellen Goheen, Alex Woods, Martin MacKenzie, John Pronos, Angel Saavedra
Front Row: Michael Murray, Dave Shaw, Leif Mortenson, Terry Shaw,



Group 2

Top Row: Bill Jacobi, Bob Edmonds, Kim Camilli, Richard Reich
Middle Row: Betsy Goodrich, Megan Dudley, Bob Schlub, Paul Zambino, Pete Angwin, Louis Iverson
Front Row: Jessica Wright, Christy Cleaver, Jessie Glaeser, Blakey Lockman, Brian Geills, Elisa Becker



Group 3

Top Row: Mike McWilliams, Matteo Garbelotto, Pete Angwin, Angel Saavedra
Middle Row: Mahsa Khorasani, Karen Hutten, Ebba Peterson, Laura Sims, Michelle Agne, Sarah Navarro, Blakey Lockman,
Front Row: Helen Maffei, Anna Leon, Joe Hulbert, Kristen Chadwick, Yun Wu



Group 4

Top Row: David Rusch, Stefan Zeglen, John Browning,

Middle Row: Will Littke, Heather Mehl, Marcus Jackson, Maia Beh, Blakey Lockman, MaryLou Fairweather, Cassandra Swett

Front Row: Amy Ross-Davis, Mike Cruickshank, Gabriela Ritokova



Group 5

Top Row: Mike Simpson, Paul Hennon, Mike McWilliams, John Schwandt, Tyler Jones
Middle Row: Brent Oblinger, Robin Mulvey, Sarah Navarro, Nick Dudley, Blakey Lockman, Ned Klopfenstein,
Front Row: Bill Woodruff, Richard Sniezko, Dan Omdal

