# Proceedings of the 66th Western International Forest Disease Work Conference

YMCA of the Rockies, Ram's Horn Lodge
Estes Park, Colorado
June 3-7, 2019

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**Alex Woods**
**Greg Reynolds**
**Kelly Burns**
**Susanna Keriö**

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## YMCA of the Rockies, Ram's Horn Lodge
## 2515 Tunnel Road, Estes Park, CO
## June 3-7, 2019

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# PANEL: MOLECULAR TOOLS (MODERATOR: JARED LEBOLDUS)

# ANALYZING GENETIC VARIATION: WHAT CAN POPULATION GENOMICS TELL US ABOUT THE BIOLOGY AND MANAGEMENT OF OUR FAVORITE FOREST PATHOGENS?

**Patrick I. Bennett[1,2*] and Jared M. LeBoldus[2,3]**

The central dogma of molecular biology states that deoxyribonucleic acid (DNA) is transcribed to a form of ribonucleic acid known as messenger RNA (mRNA). These mRNA molecules are then translated to a string of amino acids known as a protein. These processes form the foundation of our understanding of the relationships between genotype (the sequence of nucleotides at a given locus) and phenotype (the observable characteristics resulting from the expression of a genotype in a given environment). A DNA molecule consists of four chemical residues (adenine, thymine, cytosine, and guanine) known as nucleotides. All of the variation we observe in nature reflects differences in the composition, structure, and expression of the nucleotide sequences present in DNA. Population genomics provides invaluable tools for investigating the evolution of plant pathogens by analyzing the variation in nucleotide sequences. This enables studies of phenotypic characteristics that may be relevant to managing plant diseases such as pathogenicity, virulence, and host specialization (Grünwald et al. 2016). These tools also allow for the study of basic biological and evolutionary characteristics of plant pathogens including reproduction, migration, and natural selection (Grünwald et al. 2016). The population genomics framework has particular utility for investigating the epidemiology of invasive pathogen populations including introduction event(s), dispersal, colonization history, centers of origin, and novel adaptations in new environments (Barnes et al. 2014, Garbelotto et al. 2013, Goss et al. 2009, Kamvar et al. 2015). This information is essential for developing efficient and effective plant disease management strategies.

The methodology and experimental design for population studies generally follow a framework that includes collecting samples of infected plant tissues, isolating the pathogen (if possible), extracting and purifying DNA, sequencing genomes or molecular markers, and analyzing genetic variation. With recent advancements in DNA sequencing technologies, the molecular markers used for population genomics have evolved from individual gene sequences to whole-genome data. Single nucleotide polymorphisms (SNPs) represent variation in a single nucleotide at a single locus. In population genomic studies that utilize SNPs, each isolate or individual will have a genotype consisting of all SNP loci identified across the genome. These SNP loci can only be identified by comparing individual genomes to a reference genome from a type specimen. Genetic diversity is estimated by analyzing the variation in these SNP genotypes among individuals. The spatial or geographic distribution of this genetic variation within or between pathogen populations is known as population structure.

The invasive fungal pathogen *Cronartium ribicola* causes white pine blister rust, a devastating disease of five-needle pines (*Pinus* spp.) This pathogen is a particularly good case study for demonstrating the utility of population genomics in describing the invasion and colonization history of a non-native forest pathogen. Brar et al. (2015) using SNPs studied *C. ribicola* populations in North America in order to evaluate the impacts of the human-mediated introduction and subsequent colonization on its population genetic structure. The authors sampled 76 sites in the northern United States and Canada and genotyped 1,336

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individual isolates with 31 SNP loci. Their analyses identified two distinct genetic clusters of *C. ribicola* with one cluster represented by isolates collected in northeastern North America and the midwestern United States, and the other represented by isolates collected in the western U.S. and western Canada (Brar et al. 2015). There were differences in diversity and population structure between eastern and western subpopulations that reflected the colonization histories of these regions. For example, there was greater genetic diversity in the eastern subpopulations where multiple introductions of *C. ribicola* are known to have occurred, and less diversity in the western U.S. and Canada subpopulations where one or a few introductions of the pathogen occurred (Brar et al. 2015). They also hypothesized that the absence of host species in the central U.S. and Canada could be a barrier to gene flow between these eastern and western subpopulations (Brar et al. 2015). However, their analyses revealed that some gene flow has occurred possibly due to long distance aerial spore dispersal or anthropogenic movement of infected plant material (Brar et al. 2015). The results of analyses such as these can be valuable for describing basic pathogen biology and epidemiology and can inform land management decisions that rely on knowledge of pathogen dispersal and migration (Brar et al. 2015).

Analyses of variation in SNP genotypes has also been used recently to investigate the dispersal capabilities and epidemiology of the Douglas-fir black stain root disease pathogen, *Leptographium wageneri* var. *pseudotsugae*, which is vectored by insects. The preliminary results from this study are described in Bennett et al. (*these proceedings*) and further demonstrate the utility of population genomics when studying the epidemiology of forest pathogens.

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# THE DETECTION TOOLBOX FOR INVASIVE FOREST PATHOGENS: WHERE ARE WE AT?

**Nicolas Feau[1] and Richard C. Hamelin[1]**

## Summary

In the age of the Anthropocene, trees and forests are facing unprecedented threats from Forest Invasive Alien Species (FIAS) that can cause irreversible damage to the ecosystems. There are two main reasons responsible for this threat, both of them related to human activities: the globalization of trade that triggers accidental introductions of exotic pathogens in naïve environments and the major changes in climatic conditions that are underway that may facilitate the establishment and success of introduced pathogens. The easiest and most cost-effective way to address this threat is by knowing in advance which pathogens could be introduced and become problematic and by preventing their introduction. Efficient prevention programs require the ability to rapidly detect pathogens that represent a threat with accuracy to efficiently intercept them.

Innovations in genomics and molecular biology have provided a toolbox that can address these challenges. DNA-based detection methods have the advantage of providing a quicker response than the classical way of identifying pathogens with culturing and morphological methods, and the tests can be conducted by a broader range of users since they do not require knowledge and expertise in mycology and plant pathology. For example, the DNA-barcoding method uses a short-standardized DNA marker providing a high interspecific variability and low intraspecific differences that enables the identification of organism at the species level (Hebert et al. 2003). This method has proved to be effective in forest pathogen identification (EPPO, 2016) and can be used to discriminate FIAS from species that were naturally present in an ecosystem (Comtet et al. 2015). The genetic variation comprised within the DNA barcodes has also been widely translated into taxon-specific rapid and sensitive detection assays using the polymerase chain reaction (PCR) and has been applied to FIAS detection (Vincelli & Tisserat 2008). Specifically, real-time PCR using TaqMan probes has become the golden standard in forest pathogen detection as it constitutes the most sensitive, specific and rapid method available.

The approaches mentioned above use only a small number of conserved genes or genome regions and assay specificity is achieved by designing primer and probes that target polymorphic sites (as single nucleotide polymorphisms, SNPs) within these gene regions, which allows discrimination between the target lineages and taxa. However, targeting SNPs solely in conserved gene regions makes it difficult to find discriminant fixed SNPs to design assays for closely related species and increases the risk of obtaining false positives. The increase in genomic resources brought by next generation sequencing is providing some solution to these challenges. It has made it possible to mine entire genomes of invasive pathogens and their close relatives to identify genes or genomic regions of greater discriminatory power than the conserved genes traditionally used to develop real-time PCR assays. Once identified, these unique genes can be translated in real-time PCR assays of high accuracy. We developed the GEDI approach to compare the genome of a targeted taxon to its closest relatives and identify genes and genomic regions that are unique to this taxon (Feau et al. 2018). Translating unique genes into real-time PCR assays increases the specificity of the assay by reducing the risk of false positives. Using GEDI TaqMan assays for detecting the sudden oak

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death and sudden larch death pathogen *Phytophthora ramorum* reduced the false positive rate by 4.2% compared to assays targeting conserved genome regions such as the ITS region (Feau et al. 2019).

Most of the currently available detection and identification tools enable users to identify target FIAS. However, they provide limited (if any) information on their origin and on the risk associated with their introduction in a naïve environment. The ability to determine the source of introduction would allow users to identify locations of higher risk, thus facilitating targeted interventions. Knowing the potential risk associated with the introduction of an alien or unknown pathogen would improve management decisions-making. In the BioSAFE initiative (BioSurveillance of Alien Forest Enemies - http://www.biosafegenomics.com/) we are developing a new generation of biosurveillance tool that will generate the genomic profiles of large numbers of pathogen and pest samples to return accurate identification, source assignment, and life-trait prediction (Hamelin & Roe 2019). This genomic tool is based on target enrichment of hundreds of genome regions that will provide information on the taxonomic identification and the geographical origin of the sample tested. A third category of genome regions will inform on its lifestyle and fitness traits that could impact its invasiveness. This category will cover genes related to functional traits, such as pathogenicity, the ability to infect a particular host and/or multiple hosts, and the capacity to synthetize certain enzymes and metabolites that are required for infection and pathogen lifestyle. A proof-of-concept of this tool is under development in the BioSAFE project, focusing on the sudden oak death (*P. ramorum*) and the Dutch elm disease (*Ophiostoma novo-ulmi*) pathogens (Hamelin & Roe 2019).

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# DISCOVERING KEY RELATIONSHIPS BETWEEN FOREST DISEASE / HEALTH AND MICROBIAL COMMUNITIES

**Jane E. Stewart[1], Mee-Sook Kim[2], Brad Lalande[1], and Ned B. Klopfenstein[3]**

The forest pathology paradigm is shifting to include complex ecological interactions that contribute to disease development, such as 1) microbial communities associated with the soil or host plant that suppress or enhance disease; 2) invasive pathogens that are associated with new hosts in new environments; 3) host and pathogen populations that display different adaptation and ecological behaviors; 4) induced host resistance to disease that is derived from differential gene expression; and 5) other interacting environmental factors that influence biological processes related to plant disease.

Currently, the impacts of climate change are of paramount importance, because the behavior of forest pathosystems is changing and forest diseases are occurring in an unprecedented manner. For example, native forest pathogens could behave like invasive pathogens because of climatic influences on the abiotic/biotic environment and hosts. Thus, novel and robust approaches are needed to understand and manage forest diseases caused by endemic and invasive pathogens under changing climates (Mendes et al. 2011). Forest diseases and associated microbial processes are the result of dynamic, long-term, and complex interactions among the pathogen, host, and the biotic and abiotic environment.

Recent findings suggest that single microbes are not always the sole agent responsible for forest ecological processes, such as disease, symbiosis, decomposition, and others. Instead, many forest ecological processes are likely the result of complex interactions among microbial communities. For example, biological control activities by *Armillaria altimontana* and associated microbes may act in concert to protect western white pine (*Pinus monticola*) from Armillaria root disease (Warwell et al. 2019). Furthermore, ecological communities can be strong indicators of overall forest health. Recently developed technologies, such as metagenomics (or metabarcoding), allow analyses of complex microbial communities associated with soil, organic debris, rhizosphere, root/stem/foliar endophytes, and phyllosphere in association with forest health and ecological processes (Terhonen et al. 2019).

Innovative approaches are essential for assessing the interactions and functions of the microbial communities in forest disease processes, while integrating influences of other environmental factors. Understanding the myriad of interactions among host, pathogen, microbial community, and environment requires unprecedented integration of novel methodologies (Denman et al. 2018). Results from such integrated information will help develop novel approaches to manage forest disease and improve forest health by promoting conditions that suppress disease or enhance beneficial processes.

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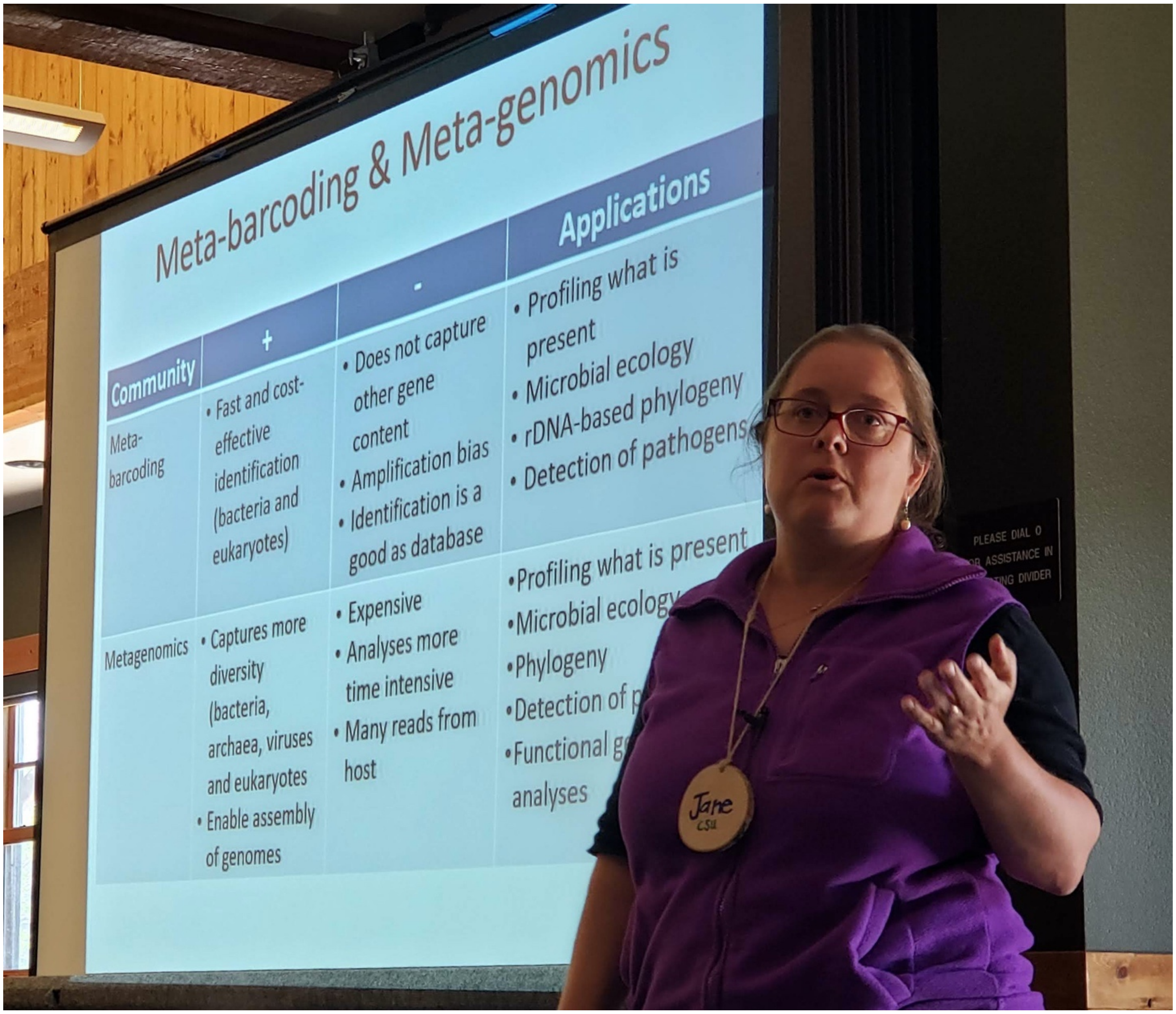

Meta-barcoding & Meta-genomics

| Community | + | - | Applications |
|---|---|---|---|
| Meta-barcoding | • Fast and cost-effective identification (bacteria and eukaryotes) | • Does not capture other gene content<br>• Amplification bias<br>• Identification is a good as database | • Profiling what is present<br>• Microbial ecology<br>• rDNA-based phylogeny<br>• Detection of pathogens |
| Metagenomics | • Captures more diversity (bacteria, archaea, viruses and eukaryotes)<br>• Enable assembly of genomes | • Expensive<br>• Analyses more time intensive<br>• Many reads from host | •Profiling what is present<br>•Microbial ecolog<br>•Phylogeny<br>•Detection of p<br>•Functional g analyses |

# USING COMMERCIAL SERVICES FOR MOLECULAR DISEASE DIAGNOSIS: FROM FIELD TO PRELIMINARY IDENTIFICATION

**Lori Winton[1] and Gerry Adams[2]**

Identification of fungi and other microorganisms suspected of causing forest diseases often requires DNA sequencing of one or more segments of the candidate genome. However, this work entails specialized training and laboratory equipment which is largely unavailable to field pathologists. Although university and research colleagues are often willing and able to help, that is not a viable solution for the national community of USDA Forest Service field pathologists. For this purpose, we explored commercial DNA Barcoding and Metabarcoding services that could accept samples fresh (or dried) from the field with no preliminary lab work.

DNA barcoding is the identification of a single organism by means of sequencing of a specific gene fragment. We found several companies that could process animal samples or pure cultures of microorganisms. In Canada and Europe, we found some companies would process fungal fruiting bodies, but none in the United States. MacrogenUSA (macrogenusa.com) expressed interest in working with us to develop an easy process with a high success rate and data quality. The application of samples to Whatman® FTA PlantSaver® Cards whilst in the field increases the chance of success but does not require special handling. Good quality samples are crushed into the paper matrix of the card and the DNA collected thereby is stable for years at room temperature. We smashed nine known samples onto FTA cards, one of which was a mushroom which had been dried for one year, and sent them to MacrogenUSA for identification. The resulting ITS (inter-transcribed spacer of ribosomal DNA) sequences were of superior quality in all cases except the one that was chosen specifically as subpar. Even so, the BLAST reports were 100% congruent with expectations and in two cases refined our morphological identifications. The price of FTA cards was approximately $8.00 each and MacrogenUSA charged $42.50 per sample for DNA extraction, PCR, ITS sequencing, and BLAST report. At just over $50.00 per sample we determined this a good value for high quality data.

For DNA Metabarcoding (using mass sequencing techniques to taxonomically describe multiple species present within a sample) we used RTLgenomics (rtlgenomics.com) to help diagnose the causal agent of the aggressive running canker disease of trembling aspen in Alaska. We requested the Earth Microbiome Project assays for Fungi, Eukaryotes, and Prokaryotes using the Illumina MiSeq platform and received raw data as well as bioinformatic analysis. In this case we sent four samples from a healthy tree and four samples from a diseased tree lesion. Each sample consisted of two cylinders (approximately 5 mm diameter by 6 mm long) of inner bark, cambium, and xylem. At $101.50 per sample the technique shows much potential. For the fungal assay, there was no amplification in the healthy aspen tree whilst the cankered sample was dominated by the yeast species *Nakazawea wyomingensis*; we have also cultured this yeast from diseased samples. The eukaryotic assay yielded 100% *Populus tremuloides* (aspen) fragments for the healthy tree on our own analysis, however the provided Kronos bioinformatics analysis identified these as *Persea schiendeana* (avocado); the diseased sample yielded 38% aspen and 62% *Nakazawea wyomingensis.* The healthy tree bacterial assay yielded 75% Halospirulina sp., 22% unknown (low confidence classification) or unclassified (missing taxonomic information in the database) bacteria; the diseased bacterial assay consisted of 53% unclassified bacteria, 24% Pseudoxanthomonas, 17% Halospirulina, and the rest were

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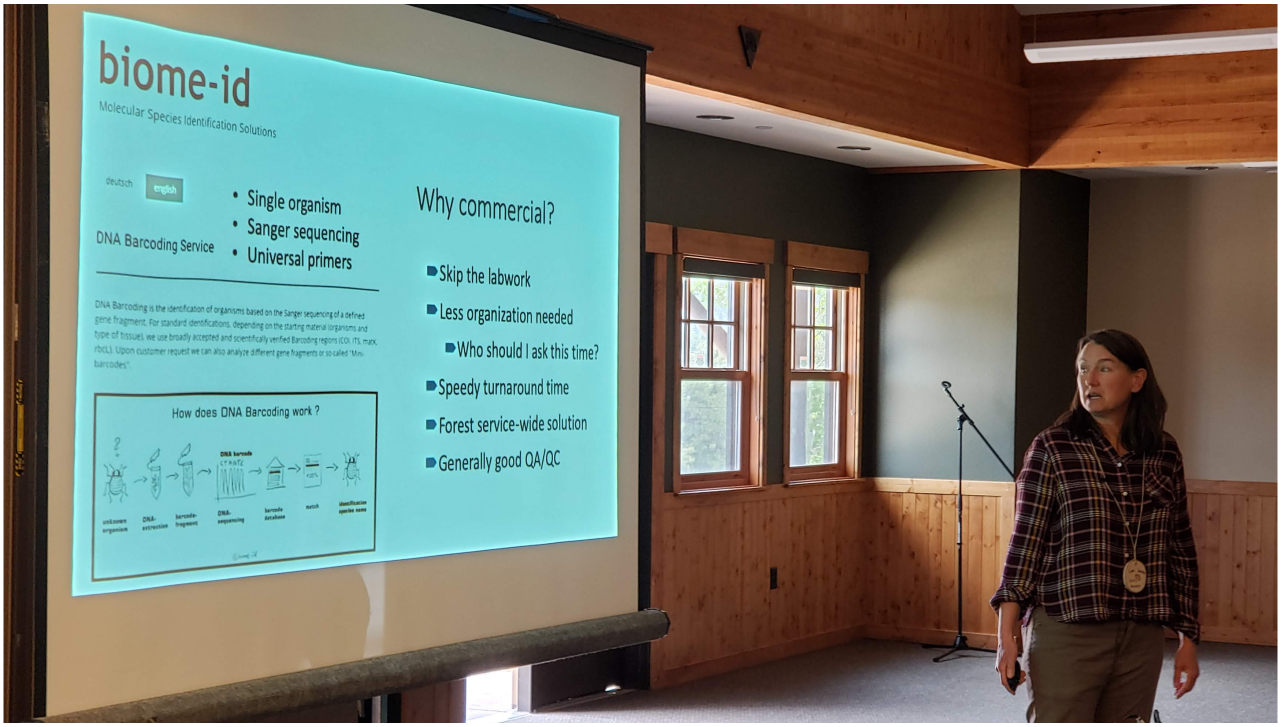
unknown bacteria. Our own analysis found that many of the unclassified or unknown fragments were actually chloroplast and mitochondrial DNA of aspen.

While we found the services and techniques of both companies very useful and cost effective for microbial identification, they should be used by those with some experience in molecular biology. Clearly one should use caution when using commercial services for molecular disease diagnosis and consider any identification provided by the company as tentative.



biome-id

Molecular Species Identification Solutions

deutsch english

DNA Barcoding Service

- Single organism
- Sanger sequencing
- Universal primers

DNA Barcoding is the identification of organisms based on the Sanger sequencing of a defined gene fragment. For standard identifications, depending on the starting material (organisms and type of tissue), we use broadly accepted and scientifically verified Barcoding regions (COI, ITS, matK, rbcL). Upon customer request we can also analyze different gene fragments or so-called "Mini-barcodes".

How does DNA Barcoding work ?

Why commercial?

- Skip the labwork
- Less organization needed
  - Who should I ask this time?
- Speedy turnaround time
- Forest service-wide solution
- Generally good QA/QC

# GENOMICS EMPOWERED APPROACHES TO PROTECTING FOREST HEALTH

**Jared M. LeBoldus[1,2*]**

Invasive fungal pathogens represent a clear and present threat to the health and sustainability of forested ecosystems in the United States. The list of diseases increases on a yearly basis, and relatively few new approaches for managing these diseases have been developed. To date, efforts have focused on quarantine, eradication, chemical/biological control, and disease resistance breeding programs. Although likely the most sustainable and effective way to manage invasive diseases following initial establishment; disease resistance breeding programs take decades to develop. Herein we present a genomics framework for the rapid identification and deployment of disease resistance to combat invasive forest pathogens. Initially, two concepts are defined: (i) genome sequencing/re-sequencing; and (ii) linkage disequilibrium mapping. How these concepts are used to identify genes associated with resistance was then outlined. *Populus trichocarpa* and *Sphaerulina musiva* were used as a case study to illustrate how this approach can be used. In this example, we combined the re-sequenced *P. trichocarpa* genome-wide association population with a disease resistance phenotyping platform to identify genes associated with resistance (Muchero et al. 2018). Finally, an argument was made for government investment in the resequencing of ecologically and economically important tree species in order to set the groundwork for a rapid genomics empowered response as new diseases emerge.

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# PANEL: STUDENT PRESENTATIONS (MODERATOR: CHRISTY CLEAVER)

# ASSESSING SOIL MICROBIAL COMMUNITIES ASSOCIATED WITH TREE HEALTH IN A WESTERN WHITE PINE (*PINUS MONTICOLA*) STAND

**Bradley M. Lalande[1], John W. Hanna[2], Marcus Warwell[2], Mee-Sook Kim[3],**
**Ned B. Klopfenstein[2], and Jane E. Stewart[1]**

At a landscape level, measurements of forest health can be a fluid statistic as it relates to the specific goals of management, which differ from stand to stand (Crann et al. 2015). As a result, analyses of trees at the individual or stand level may discover previously unknown factors that influence specific tree health metrics. Above-ground dynamics, such as productivity and foliage integrity, are common measures of tree health (Trumbore et al. 2015); however, roots and their interactions with soil and microbes may have a greater impact on tree health than the above-ground dynamics.

Soil acts as a substrate for trees to grow, while microbial communities associated in the rhizosphere and adjacent soil facilitate water and nutrient uptake (Baldrian 2017, Crann et al. 2015, Lee Taylor & Sinsabaugh 2014, Robertson & Groffman 2014). Additionally, microbes can act to impede the establishment of harmful pathogens (Baumgartner & Warnock 2006, Mesanza et al. 2016, Xiong et al. 2017, Warwell et al. 2019). Currently, root diseases are hypothesized to cause the largest damage in forests of the United States (Krist et al. 2014). The assessment of soil microbial communities will increase our awareness of the soil ecosystem associated with tree health, while increasing information for root disease management. The goal of our research was to identify how soil microbial communities differ among tree health statuses (healthy, declining, and dead) within a western white pine (*Pinus monticola*) plantation in the Priest River Experimental Forest of northern Idaho. The research question was to evaluate if soil microbial communities shift in association with changes in tree health. This study occurred in a stand historically infected by two *Armillaria* species, *Armillaria solidipes* which is well-known primary pathogen in many conifer species and *A. altimontana*, a species that appears to not harm western white pine while providing protection from pathogenic *A. solidipes* (Warwell et al. 2019).

Sixty-three trees were selected based on the historic tree health status, from a full inventory conducted in 1987 sampled in 2016. In total, 38 trees were healthy, 12 were declining, and 13 were dead. Soil samples were collected in association with each tree and DNA was extracted. Soil DNA was sequenced at the internal transcribed spacer (ITS2) (fungal) and 16S ribosomal (bacterial) DNA regions to determine microbial communities associated with each tree health status using the MiSeq Illumina platform.

Slight fungal community differences were observed between soils associated with declining and dead trees ($p = 0.055$), while no differences were observed between healthy and dead trees ($p = 0.18$) (Figure 1). Soils associated with dead trees had the greatest fungal diversity and richness within samples in comparison to healthy and declining trees (Table 1). Additionally, soils associated with healthy trees had a greater proportion of Hypocreaeceae (e.g., *Trichoderma* spp.), which can act as biocontrol agents in disease-suppressive soils.

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***Table 1:*** *Richness and diversity indices for soil microbial communities associated with tree health.*

| | Richness | Shannon | Inverse Simpson |
|---|---|---|---|
| **Fungi** | | | |
| **Healthy** | 216 ± 11.1 | 3.26 ± 0.074 | 13.1 ± 1.00 |
| **Declining** | 182 ± 24.5 | 3.05 ± 0.162 | 10.0 ± 2.21 |
| **Dead** | 243 ± 18.0* | 3.45 ± 0.119* | 15.7 ± 1.62* |
| **Bacteria** | | | |
| **Healthy** | 224 ± 15.2 | 4.29 ± 0.205* | 27.32 ± 4.06* |
| **Declining** | 217 ± 35.4 | 4.16 ± 0.476 | 22.45 ± 9.45 |
| **Dead** | 177 ± 33.8 | 3.30 ± 0.321 | 9.08 ± 6.37 |

*Identifies a significant difference (p = 0.05)

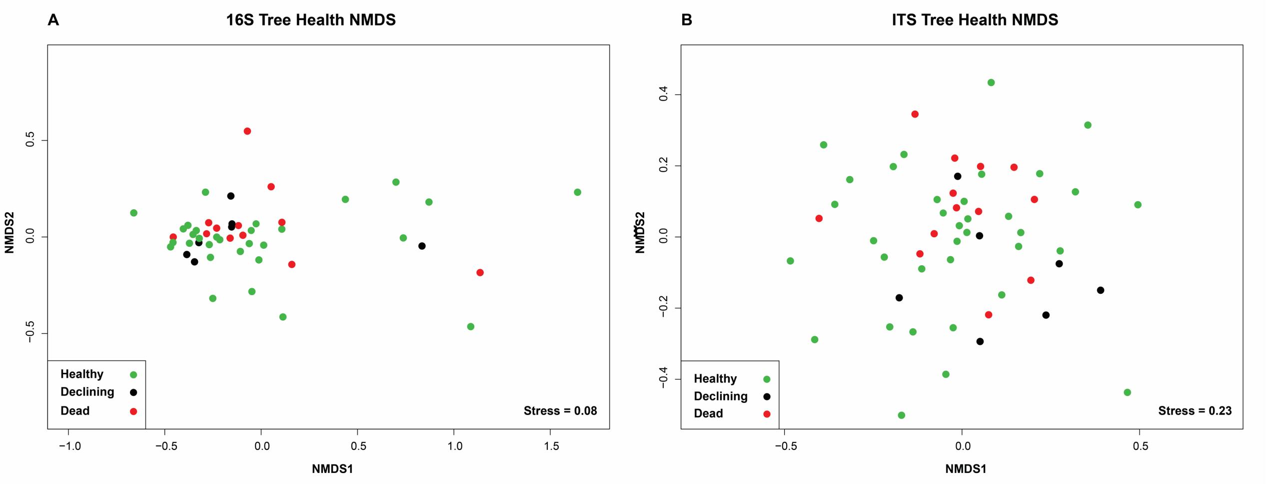

***Figure 1:*** *Non-metric multidimensional scaling plots used to determine dissimilarities between soil microbial communities associated with tree health. Tree health status for each tree is indicated by specific colored dots (Healthy = green; Declining = black; Dead = red). A) Determined high overlap between 16S (bacterial) soil communities associated with tree health with some outliers for each tree health. B) Identified similar, high overlap, between ITS (fungal) soil microbial communities associated with tree health.*

Minor dissimilarity occurred between soils associated with dead trees and healthy (p = 0.02), indicating slight differences in bacterial communities, while no differences were observed between healthy and declining trees (p = 0.88). Although no differences were observed in the overall community structure, clear patterns were found when examining the most abundant bacterial taxa (Figure 2). Soils associated with healthy trees had the greatest bacterial diversity and richness in comparison to declining and dead trees, with dead trees having the lowest diversity and richness (Table 1). Soils associated with healthy trees had a greater abundance of Chthoniobacteraceae, Nitrosomanadaceae, Acidobacteria subgroups 2 & 6, and Xanthobacteraceae, all of which function to increase soil health. Interestingly, a greater abundance of Pseudomonadaceae and Enterobacteraceae was associated with dead trees, and these organisms are important in biocontrol against pathogens in disease-suppressive soils.

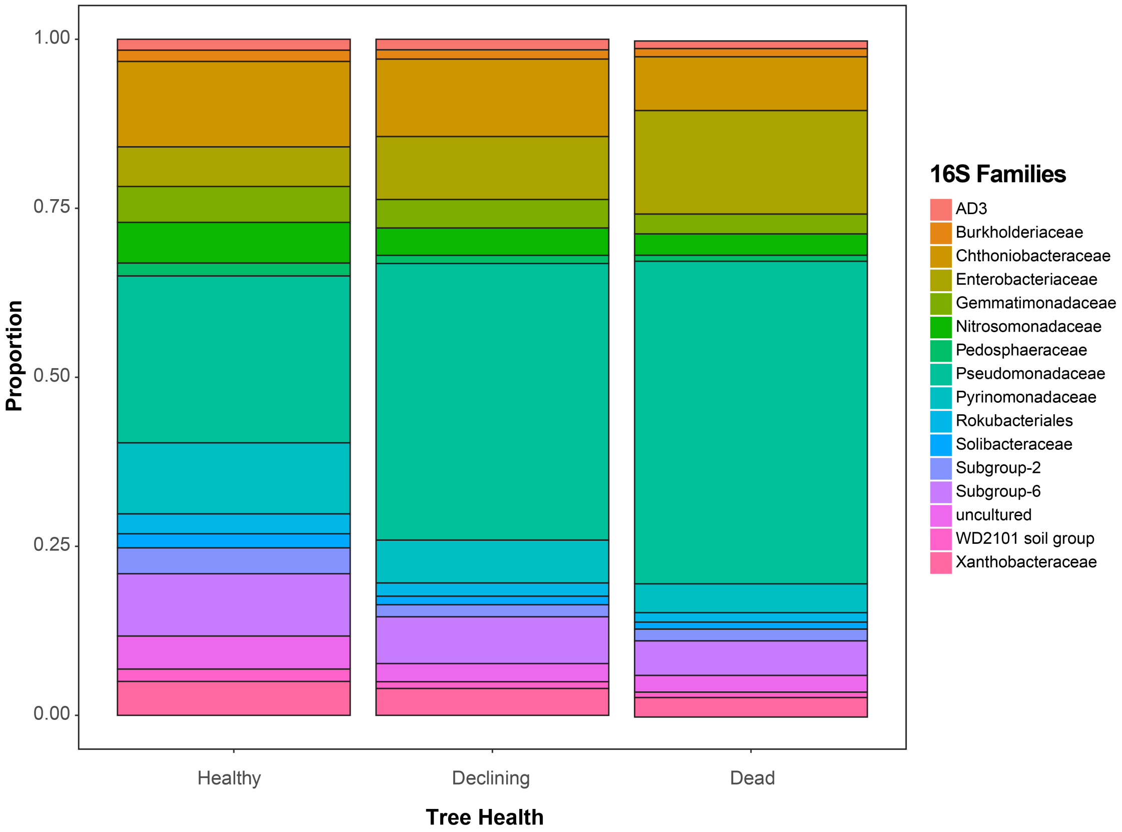

***Figure 2:*** *Bar graph determining the most abundant bacterial (16S) families associated with each tree health.*

To further determine factors that may influence soil microbial communities, soil properties were assessed at each tree location. The most significant soil property correlated to fungal community composition was pH (p = 0.001), while pH (p = 0.015) and moisture (p = 0.010) were also significantly correlated to bacterial community composition. Additionally, carbon (p = 0.001, p = 0.0232), nitrogen (p = 0.017, p = 0.01), and organic matter (p = 0.0003, p = 0.007) may have an indirect influence on microbial communities as these factors showed a significant relationship to soil pH and/or moisture.

Further analyses are needed to fully determine how soil microbial communities interact with tree health status. Functional characteristics may elucidate this relationship because many microbes are functionally redundant within the soil. Ideally, these studies will increase our knowledge of the dynamics of soil microbial communities with the goal to uncover novel approaches for managing tree health by managing forest soils to favor beneficial soil microbes that suppress root diseases or perform other beneficial functions.

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# MULTI-GENE PHYLOGENIES SHOW A CLUSTER OF *LOPHODERMELLA* PATHOGENS (RHYTISMATACEAE) AND THEIR CLOSE RELATIONSHIP WITH *LOPHOPHACIDIUM DOOKSII* (PHACIDIACEAE)

**Jessa P. Ata[1,2], Kelly S. Burns[3], Suzanne B. Marchetti[4], Isabel A. Munck[5],**
**James J. Worrall[4], and Jane E. Stewart[1]**

The impact of needle diseases in some conifer stands has increased worldwide due to climate change, and in the US, needle casts have become more aggressive in various native pine forests. *Lophodermella concolor* and *L. montivaga* under Rhytismataceae are two co-existing needle pathogens in lodgepole pine that caused locally severe needle cast epidemic in Gunnison National Forest, CO from 2008-2012 (Worrall et al. 2012). *Lophophacidium dooksii,* on the other hand, contributed to the white pine needle damage in the Northeastern US (Broders et al. 2015). Using morphological characterization, both taxa have been previously thought to be in separate orders – Phacidiales and Rhytismatales – within the class Leotiomycetes. However, recent genetic research suggested a closer relationship between the two taxa (Laflamme et al. 2015). Ekanayaka et al. (2019) then transferred *Lophophacidium dooksii* from Phacidiaceae to Rhytismataceae. Understanding the phylogenetic relationship of needle pathogens is important to have insights on pathogen evolution and lifestyles. This research aimed to understand the phylogenetic relationships of *Lophodermella* species with other Rhytismataceae species, and with *Lophophacidium dooksii* through multi-gene analyses, particularly the use of internal transcribed spacer region and 5.8S ribosomal RNA (ITS), large subunit ribosomal nucleic acid (LSU), and translation elongation factor (TEF1-a). Morphological characterization showed subhypodermal hysterothecia as a common morphological character among the two genera while only slight differences in size and shape of asci were observed (Figure 1). However, ascospore shape varies between *Lophodermella* species and *Lophophacidium dooksi*. Interestingly, the inability of *L. montivaga, L. concolor,* and *Lophophacidium dooksii* to grow in artificial culture media may suggest an obligate biotrophic lifestyle on their pine hosts. Individual phylogenies of the three loci that include *Lophodermella montivaga* and *L. concolor* from *Pinus contorta*, and *Lophodermella* sp. and *L. arcuata* from *P. flexilis*, and *Lophophacidium dooksii* from *P. strobus* are congruent, whereby the three species form a single clade for each phylogeny, and the phylogeny of combined ITS-LSU-TEF1alpha dataset showed a well-supported clade with 1.0 Bayesian posterior probability and 95 percent bootstrap support (Figure 2). The subcluster within the *Lophodermella* clade also suggested a closer relationship between *L. montivaga* and *Lophophacidium dooksii* along with *L. arcuata* and *Lophodermella* sp. This study supports the idea of a *Lophodermella* cluster within Rhytismataceae and provides further evidence that *Lophophacidium dooksii* is member of the Rhytismataceae family. This study also highlights the importance of molecular tools as part of an integral approach for needle fungal pathogen taxonomy. This study further recommends genetic characterization of other *Lophodermella* and *Lophophacidium* species to provide a higher phylogenetic resolution.

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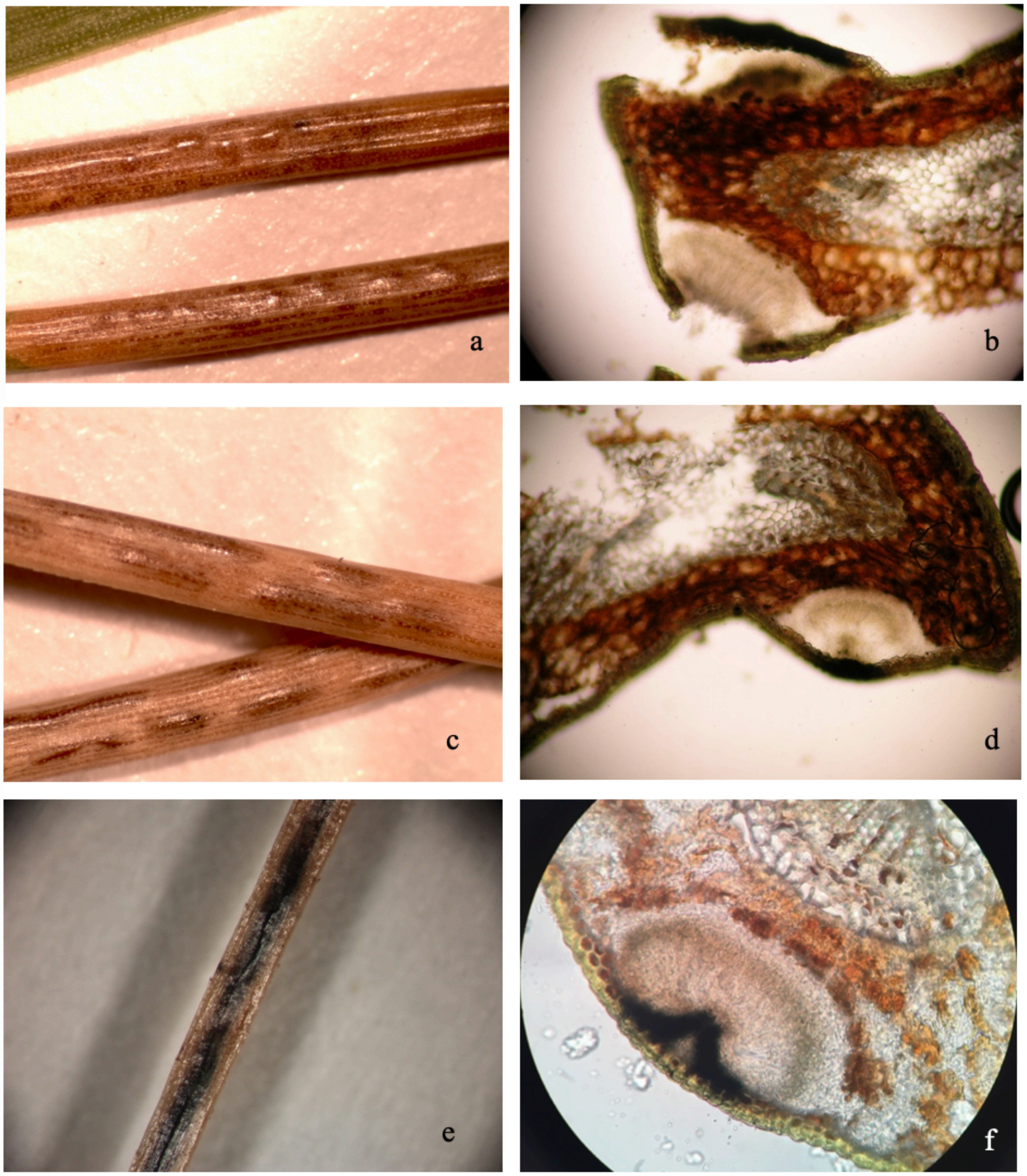

***Figure 1:*** *Morphological characteristics of* Lophodermella concolor *(a, b),* L. montivaga *(c, d) on lodgepole pine, and* Lophophacidium dooksii *on white pine (e, f). Concolorous hysterothecia of* L. concolor *are short and elliptical (a), subhypodermal, deeply seated hysterothecia of* L. concolor *(b), brown long elliptical hysterothecia of* L. montivaga *(c), subhypodermal ascoma of* L. montivaga *(d), dark linear subhypodermal hysterothecia of* Lophophacidium dooksii *(e and f).*

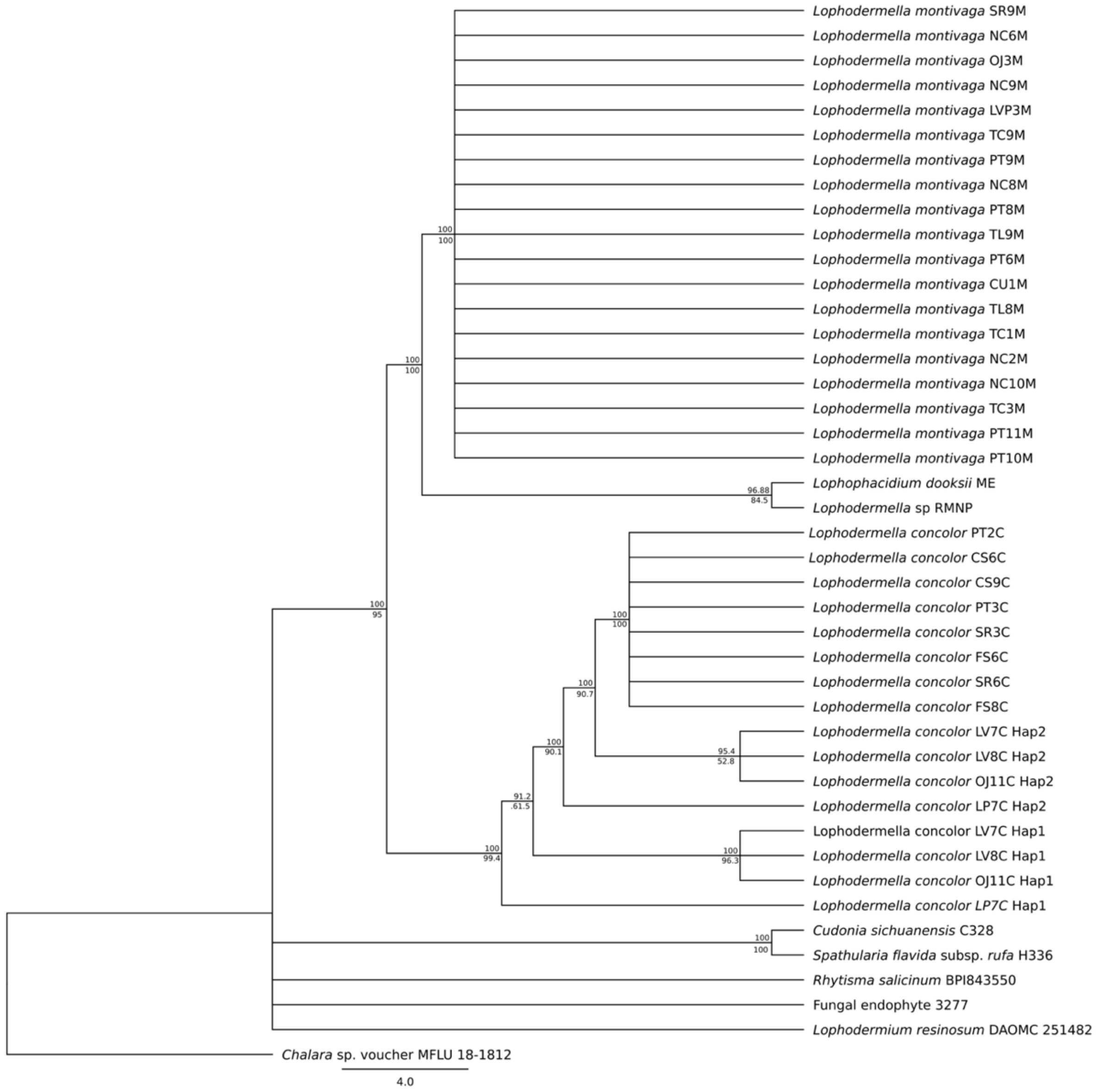

***Figure 2:*** *Maximum likelihood phylogeny depicting phylogenetic relationships of* Lophodermella montivaga *and* L. concolor *with other Rhytismataceae species based on combined dataset (ITS-LSU-TEF1a). Posterior probabilities greater than 0.80 and bootstrap support values greater than 50 are shown above and below node, respectively.*

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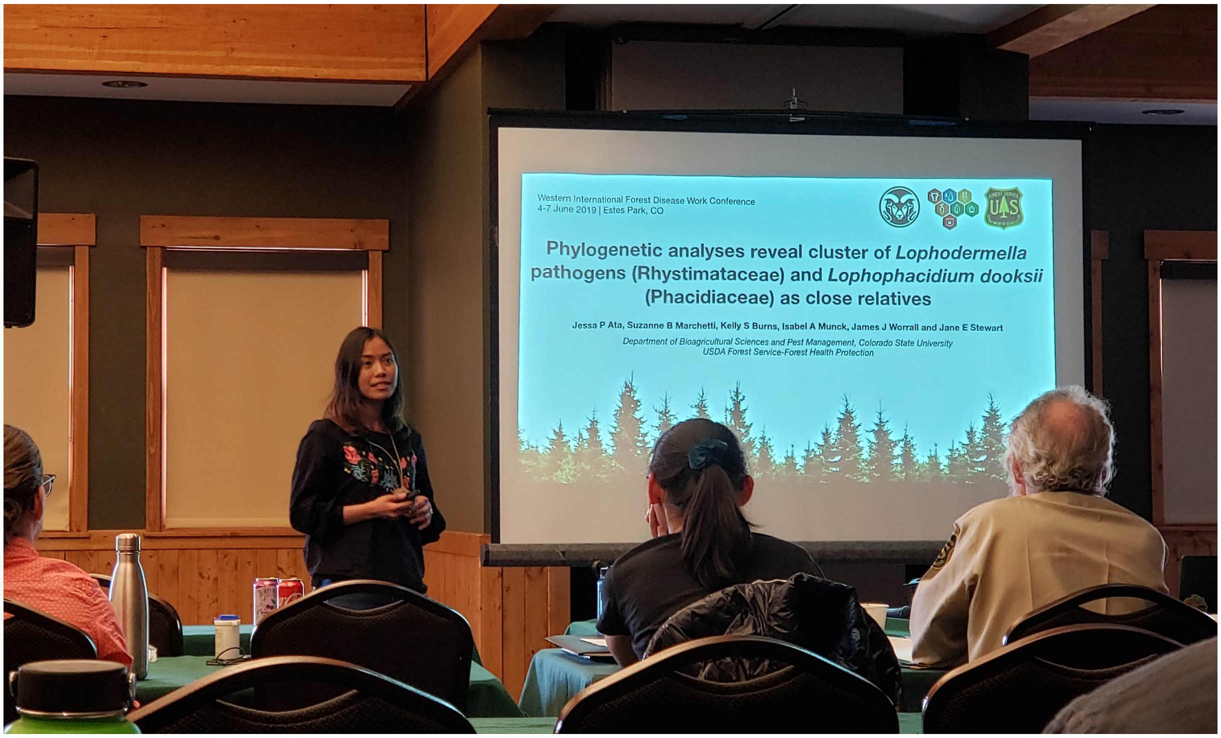

# IDENTIFYING GENOMIC PATTERNS ASSOCIATED WITH THE ECOLOGICAL ROLES OF FUNGI: DEVELOPMENT OF A FUNGAL LIFESTYLE DATABASE AND APPLICATION OF MACHINE LEARNING

**Erika Dort[1], Elliot Lane[2], Nicolas Feau[1], Mathieu Blanchette[2],**
**and Richard Hamelin[1]**

Fungi display a large diversity of ecological roles and lifestyles that influence their biological interactions within the ecosystems in which they reside. These diverse interactions have important implications for a number of fields, including forestry and agriculture where fungi often cause significant damage as plant pathogens. One major challenge in fungal plant pathology is the difficulty of predicting the potential threats posed by unknown or undescribed species; we are proposing a genomics approach to address this challenge. With now close to 1300 sequenced fungal genomes, the Joint Genome Institute's MycoCosm Research Portal (Grigoriev et al. 2014) provides researchers with an unprecedented opportunity to analyze the genetic basis of fungal life traits on a large scale. We created a database using the genomic data available from MycoCosm for 436 species spanning four ascomycete classes (Dothideomycetes, Sordariomycetes, Leotiomycetes, Pezizomycetes) and two basidiomycete groups (Pucciniomycotina, Agaricomycotina), including phytopathogenic species that are relevant to forestry. Using the information from both MycoCosm and literature searches, we categorized each species into its respective lifestyle(s) and identified important biological traits for the plant-pathogenic species. In total, we classified species into 24 unique categories spanning from plant-interacting lifestyles such as saprotrophs, necrotrophs and biotrophs, to bioenergy-related fungi like thermophiles and acidophiles. We analyzed patterns among our selected species on the basis of four categories of biological molecules known to play important roles in plant-pathogen interactions: 1) secondary metabolite clusters – includes important phytotoxins (Fox & Howlett 2008), 2) carbohydrate active enzymes – involved in the modification of plant and fungal cell walls (van den Brink & de Vries 2011) and host infection processes (Lyu et al. 2015), 3) peptidases – involved in nutrient assimilation and secreted as effectors during infection (Muszewska et al. 2017, Krishnan et al. 2018), and 4) membrane transport proteins – involved in host-nutrient uptake, protection against plant defense compounds and secretion of phytotoxins (Del Sorbo et al. 2000). We used principal component analysis (PCA), followed by a machine-learning approach to identify fine-scale patterns associated with specific plant-interacting lifestyles. Our PCA results show that obligate biotrophs share genomic profiles for carbohydrate active enzymes (Figure 1), and the preliminary machine-learning results from the secondary metabolite clusters support this obligate biotroph profile (Figure 2). Our results have important implications for predicting life traits in unknown or undescribed fungi, and we believe that this approach can help streamline risk assessment and increase our understanding of important biological traits relevant to mitigation of disease outbreaks.

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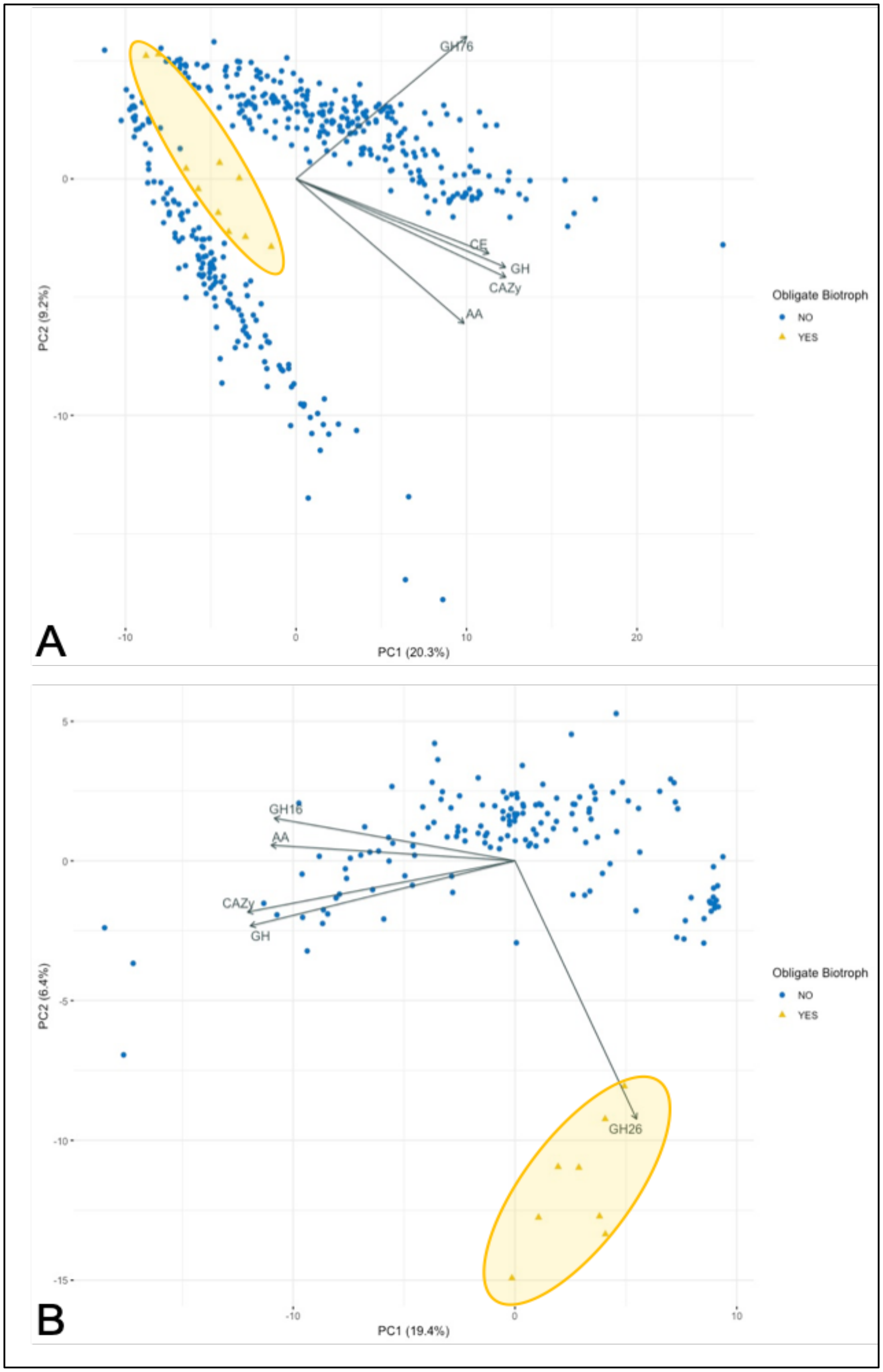

***Figure 1:*** *Principal component analyses performed on genome data for carbohydrate active enzymes (CAZys) from the Basidiomycota and Ascomycota species combined (A), and only the Basidiomycota species (B).*

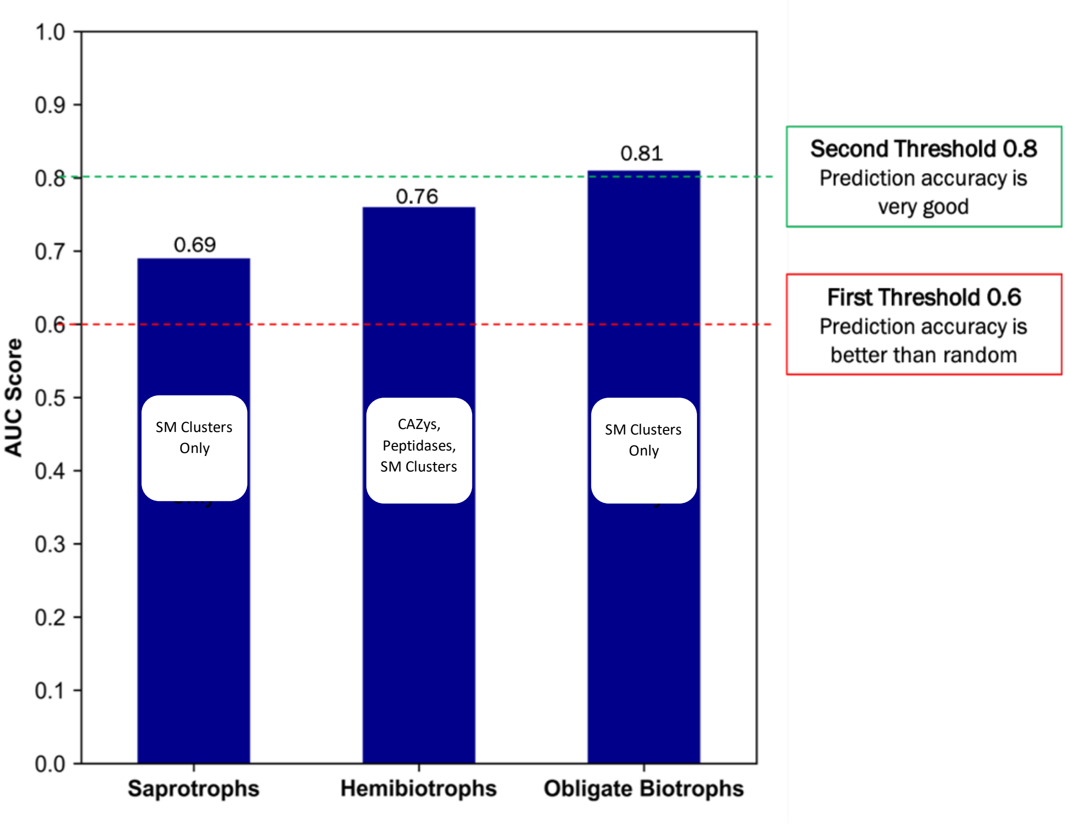

***Figure 2:*** *Machine learning (ML) prediction results from three plant-interacting lifestyles that exhibit prediction accuracies above the two important AUC (a measure of prediction accuracy) threshold values used in machine learning: 0.6 and 0.8. The AUC scores for saprotrophs and hemibiotrophs are above the first threshold (0.6) indicating that machine learning is able to predict those lifestyles with accuracy that is better than random, and the AUC score for obligate biotrophs is above the second threshold (0.8) indicating that machine learning is able to predict that lifestyle with consistent accuracy.*

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My Background

University of Victoria

- BSc Biology *(Minor in Environmental Science)*
- MSc Biology *(Fungal Molecular Genetics)*

**Thesis:** Reprogramming the expression of a dsRNA mitovirus from the mitochondria to the cytoplasm in the Dutch elm disease pathogen, *Ophiostoma novo-ulmi*

**MSc:** pairing *Ophiostoma novo-ulmi* cultures for mycovirus transfer

- Viticulture Certificate

**Viticulture Program:** visiting a vineyard in the Okanagan (BC)

THE UNIVERSITY OF BRITISH COLUMBIA

- PhD Forestry *(Forest Pathology and Genomics)*

# SUDDEN OAK DEATH MANAGEMENT IN SOUTHWEST OREGON

**Hazel Daniels[1] and Jared LeBoldus[1]**

*Phytophthora ramorum* is the oomycete pathogen responsible for Sudden Oak Death (SOD) in California and Oregon forests. It was first detected in Curry County, Southwest Oregon in 2001. This discovery prompted the creation of a multi-agency management team which has been treating SOD infestations in the area since that time. Prior to 2011, the focus of Oregon management was complete eradication of all disease centers through removal of infected plant material. A Generally Infested Area (GIA) was established in 2012 when infestations outpaced funding for treatment. Despite intense eradication procedures, the disease continued to spread. Current Oregon management focuses on reducing the spread of SOD within Curry County and preventing spread to neighboring counties. The majority of previous research has focused on NA1, previously the only clonal lineage located in Curry County. The EU1 lineage was first confirmed in Curry County forests in 2015. Therefore, there's a need to reassess the effectiveness of management techniques with the discovery of this more virulent lineage.

This study re-evaluates the efficacy of existing SOD management strategies in Oregon since the introduction of the EU1 lineage, and asks the question: Are current treatments effectively reducing *P. ramorum* infestations in soil and vegetation for both NA1 and EU1 lineages? Based on the success of previous treatments on NA1 spread within the quarantine zone, it is hypothesized that current management is reducing residual *P. ramorum* infestations in soil and vegetation for EU1 as well. In addition to sampling treated NA1 and EU1 sites, samples are also collected from untreated NA1 and EU1 sites, and completely uninfested sites which stand as negative controls. Additionally, impacts of variable intensity natural fire on eradication of NA1 can be studied through the 2017 Chetco Bar Fire, which swept through portions of the Oregon Quarantine Zone and GIA. Soil and vegetation samples will be collected, processed, and cultured isolates of *P. ramorum* from these samples will be sequenced to confirm their lineage. One-way ANOVA and logistic regression analyses will be performed on the data to demonstrate whether existing treatments still work for both EU1 and NA1 infestations, and to determine if there are any specific factors which increase or decrease the chance of successful reduction of residual SOD infestation in soil and vegetation. Preliminary data suggest that treatment is effective in reducing residual *P. ramorum* infestations in vegetation for both EU1 and NA1. It is vital to periodically assess the efficacy of disease management strategies so that they may be changed as needed to keep up with shifts in pathogen behavior and range.

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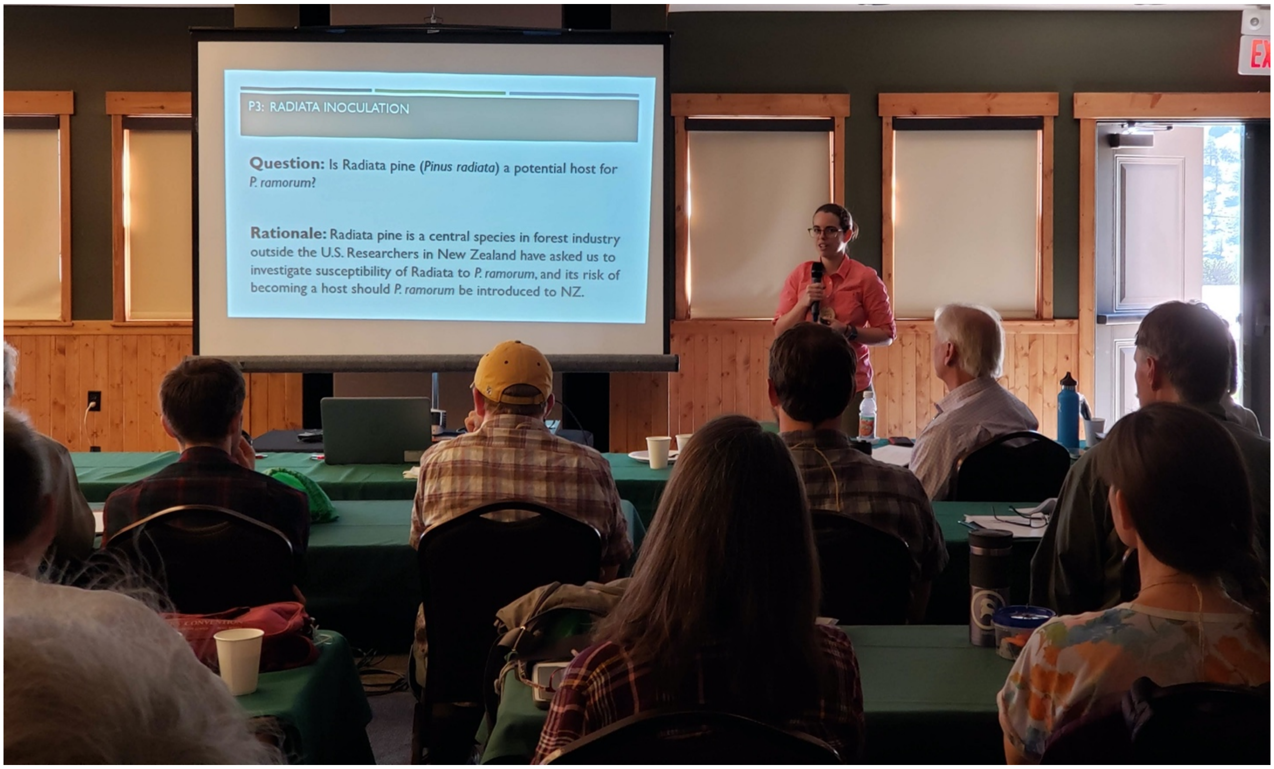
[1] *Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR*



P3: RADIATA INOCULATION

**Question:** Is Radiata pine (*Pinus radiata*) a potential host for *P. ramorum*?

**Rationale:** Radiata pine is a central species in forest industry outside the U.S. Researchers in New Zealand have asked us to investigate susceptibility of Radiata to *P. ramorum*, and its risk of becoming a host should *P. ramorum* be introduced to NZ.

# PANEL: SWISS NEEDLE CAST (MODERATOR: HARRY KOPE)

# THE SWISS NEEDLE CAST COOPERATIVE RESEARCH AND MONITORING PLOT NETWORK: MULTIPLE OPPORTUNITIES FOR RESEARCH

**Gabriela Ritóková[1], Doug Mainwaring[1], and Dave Shaw[1]**

## Introduction

Swiss needle cast (SNC), a foliar disease affecting Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the Pacific Northwest, is caused by the ascomycete fungus *Nothophaeocryptopus gaeumannii*. (T. Rohde) Videira et al. Disease severity and growth impacts are assessed using the number of years of retained foliage. Growth declines occur due to foliar loss, and previous analyses have shown that stands averaging 1-year of foliage retention typically exhibit growth losses exceeding 50% (Figure 1). In 2013, the installation of a network of research and monitoring plots was initiated, eventually totaling 106 plots located from the Oregon-California border to SW Washington and within 35 miles of the coast. These plots were envisioned to provide sites for numerous investigations, including regional monitoring of the extent and severity of SNC, the effect of the disease on Douglas-fir growth and stand dynamics, determination of how infection varies with environmental variables, and its connection to soil or foliar chemistry (Lan et al. 2019). Among the objectives of our research was to confirm the relationship between foliage retention and disease severity and determine how this relationship varies with elevation. A second objective was to determine the relationship between SNC infection levels and Douglas-fir cubic volume growth, based on the remeasurement data collected from 66 permanent plots remeasured thus far (0.08 ha).

## Methods

The SNC Cooperative research and monitoring plot network was established between the California border and SW Washington (338 miles) and 35 miles inland from the coast in the fall of 2013, 2014, and 2015 (Figure 2). Precipitation and temperature vary across the region, with annual precipitation ranging from 1200 - 4800 mm, primarily from October through May, and mean annual temperature ranging from 13 - 18°C. The elevation of plots making up the network ranged from 40 – 800 meters above sea level.

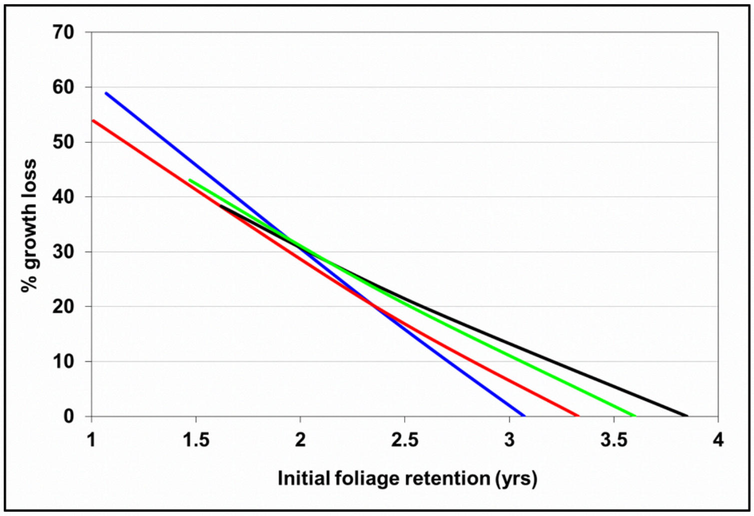


***Figure 1:*** *The relationship observed between needle retention and percent volume-growth loss in a ten-year growth impact study of 76 Douglas-fir stands in north coastal Oregon. Each line represents the needle retention-growth loss relationship during a specific measurement period. In general, growth loss peaks at about 50-60% and no growth loss is expected when trees retain more than 3-4 needle cohorts.*

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[1] *SNCC, Forest Engineering, Resource, & Management, Oregon State University, Corvallis, OR*

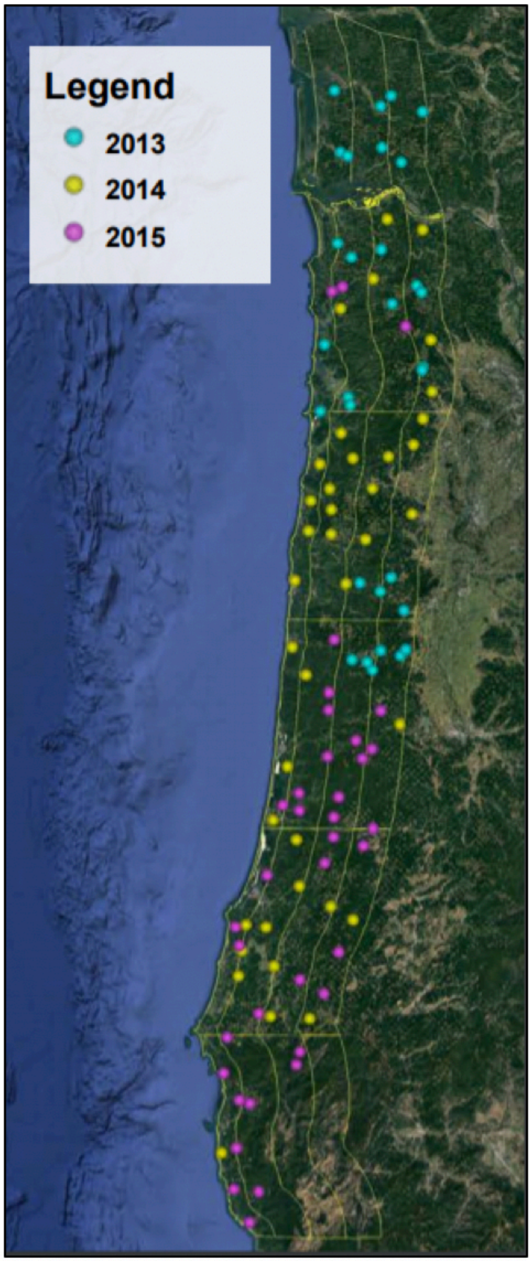

***Figure 2:*** *SNCC plot network; Sampling by year.*

## Field Methods

Foliage samples were collected on each research plot just prior to budbreak (March-May) in the spring following plot installation (2014, 2015, and 2016). In each plot, foliage samples were collected from the south side of the mid-crown of the 5 – 10 largest (by dbh) undamaged trees. Foliage samples were transported to the lab for pseudothecial occlusion counts. Foliage retention was evaluated in the field by estimating the number of annual cohorts of foliage remaining on the 4-year-old lateral branch (Maguire et al. 2011) (nearest 10% for each needle cohort). Sixty-six of the 106 plots were remeasured in fall of 2018 and 2019 after 4 – 5 years of growth. The remaining plots will be remeasured in fall 2020.

## Laboratory Methods

### *Pseudothecial Occlusion*

The degree of fungal colonization was determined visually by estimating the percent of stomates occluded by *N. gaeumannii* pseudothecia on each needle. For each selected tree, 50 needles were selected from three 2-year-old cohorts (branchlets) of each 4-year lateral branch. The needles were taped to an index card, and each needle was visually inspected with a dissecting microscope to determine proportion of needles with stomates occluded by pseudothecia, referred to as SNC incidence. The proportion of pseudothecia emerging from stomata (pseudothecial density) were recorded for the first 10 needles with positive incidence. Pseudothecial density was visually observed and averaged in three randomly chosen locations (tip, middle, and base) of each of the 10 needles, following methodology described by Manter et al. (2000) and Winton et al. (2002). The SNC disease severity index was calculated by multiplying the percentage of occluded stomata (pseudothecial density) by incidence of needles with pseudothecia (Manter et al. 2000).

## Results and Discussion

The series of graphs confirmed the positive relationship between foliage retention, disease severity index, and elevation (Figures 3, 4). With the Coast Range running parallel with the coast, increased elevation is associated with greater distance from the coast and from areas previously identified as being particularly affected by SNC (Hansen et al. 2000). Greater elevations are also associated with cooler winter temperatures that have been hypothesized to slow over-winter pseudothecial development (Manter et al. 2005, Ritóková et al. 2016), decreases in soil nitrogen that have been found to be associated with greater foliage retention (Hatten et al. 2018), and greater summer temperatures associated with continentality and reduced SNC levels (Zhao et al. 2011).

A stand-level regression analysis of the growth of these plots correlated periodic annual cubic volume growth with Douglas-fir basal area, basal area of other conifers, basal area of hardwoods, an SNC-adjusted estimate of site index (Bruce 1981), and average stand foliage retention. Estimates of site index based on height-age pairs were adjusted by an equation correlating foliage retention and height increment (*unpublished*). The implied cubic volume growth loss due to high SNC (low foliage retention) was estimated to be as high as 40% when foliage retention was as low as 1.0 years (Figure 5). This maximum growth loss estimate was not as high as estimates based on the volume growth-foliage retention relationships established during four separate growth periods between 1998 and 2008 on a previous set of permanent plots. Maximum growth losses estimated during the 1998-2008 growth periods exceeded 50%. The smaller value from the current analysis may be due to a difference in the sampled population. In the time between the original 1998 plot establishment and the 2013 establishment of replacement plots, many underperforming pre-merchantable coastal stands have been harvested and replanted to western hemlock. As a result, it is believed that areas particularly subject to the highest levels of SNC no longer have significant Douglas-fir stands in the target age range, and are thus not represented in the sampled population.

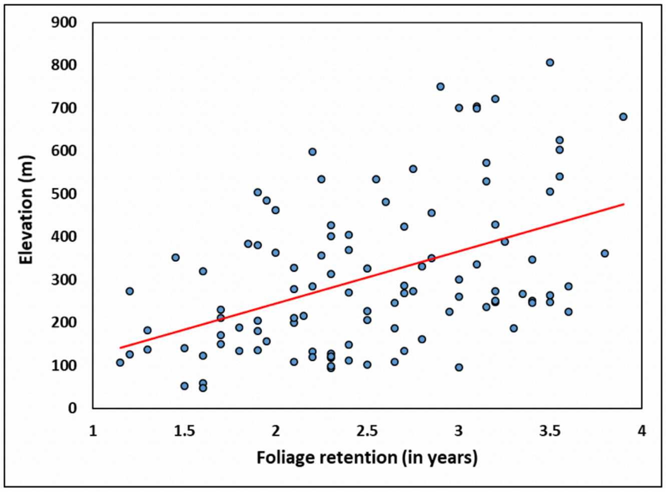

***Figure 3:*** *Trend in plot average foliage retention with elevation, with the highly variable positive relationship reflecting the confounding and interactive effects of topography and climate.*

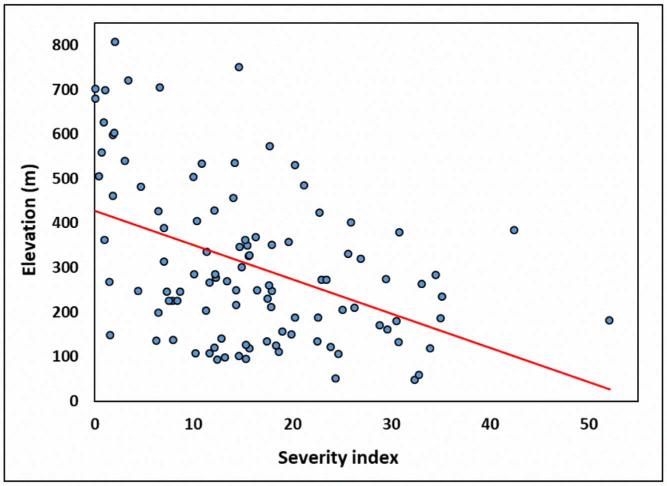

***Figure 4:*** *Relationship between severity index and elevation.*

If severity index is included as a covariate in the stand level regression analysis, it is also a significant predictor, indicating the degree to which pseudothecial occlusion is the driving force in growth losses. Foliar loss is the result of extreme occlusion, but partially occluded needles remain only partially functional as gas exchangers. At a given level of foliage retention, there is large variation in the degree of pseudothecial occlusion (Figure 6), and the two factors together are related to cubic volume growth loss (Figure 7).

***Figure 5:*** *Relationship observed between cubic volume growth loss and foliage retention, 2013-2019.*

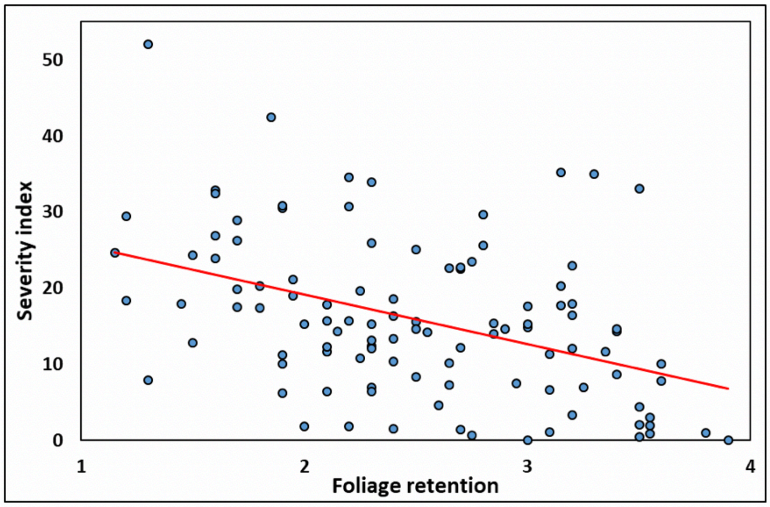

***Figure 6:*** *Estimated relationship between mean SNC disease severity index and foliage retention at mid-crown level in young Douglas-fir plantations.*

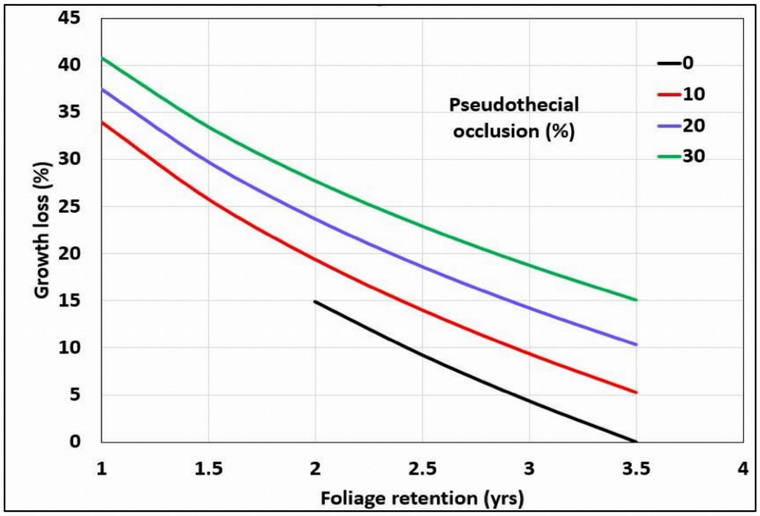

***Figure 7:*** *Predicted relationship between foliage retention, pseudothecial occlusion and cubic growth loss.*

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Foliage rete
Plot network consis
106 plots
...and a real nice
foliage retention
distribution


Early 1900's
Europe, 1st described in Exotic Plantations of DF
Considered unimportant in North America
1950'-1970's
Introduced to Chile?, New Zealand
1970/1980's
Xmas Trees in OR and WA
1st noted in Tillamook Basin, OR
1990's
Emerges as a major foliage disease of Douglas-fir plantations in Oregon (and Wash) w/in 50 km of the Coast
Major research initiative begun at OSU, including BPP: Everett Hansen, Jeff Stone, Dan Manter, Lori Winton, Paul Reeser, Pablo Rosso, Bryan Capitano et al.
2000 – present
Intensification within epidemic area
Marginal movement east, flare-ups in Cascade valleys
Intensification in WA and BC?
Absent as a major disease in N. California
Time line for SNC
SNC needle casting in Tillamook Basin, Oregon

# SWISS NEEDLE CAST IN WESTERN WASHINGTON, 1998-2018

**Daniel Omdal[1] and Amy Ramsey[1]**

## Introduction

The fungus that causes Swiss Needle Cast (SNC), *Nothophaeocryptopus gaeumannii*, is found throughout the range of its only host, Douglas-fir (Shaw et al. 2011). Swiss Needle Cast causes premature foliage loss and defoliation and can reduce growth of host trees, alter wood properties, and affect stand structure and development (Johnson et al. 2005, Maguire et al. 2011, Weiskittel et al. 2006). In Washington, SNC is most prevalent in coastal forests due to a fungi-favorable climatic (mild winters and wet springs and summers) and topographic conditions. Efforts to measure the incidence and severity, as well as detect and map the distribution of SNC, in coastal Washington have been ongoing since 1997. Aerial surveys have been conducted in coastal Washington in 1998-2000, 2012, 2015, 2016 and 2018, and ground surveys have been occurring nearly annually since 1997.

## Methods

Aerial detection surveys are an efficient and economical method of collecting and reporting data on forest insects, diseases, and other disturbances. Douglas-fir infected with *N. gaeumannii* may exhibit yellowing and browning of infected needles, which presents a unique aerial survey signature, or color variance, that is most prominent for ca. 6 to 8 weeks prior to bud break. Aerial survey for SNC in Washington is timed to occur when the crown color symptoms have developed, but before the new foliage has emerged (bud break) in late spring. The SNC survey area in Washington extends from the Columbia River north to the Strait of Juan de Fuca, and from the coastline eastward, until the SNC signature is no longer observable.

Anywhere from 30 to 75 forest management units (FMU) have been included in annual SNC ground surveys. Stand color, landscape position, elevation, aspect, and average tree age are recorded for each site. Needle retention, diameter at breast height, and crown color are recorded for ten trees along a ca. 100 M transect at each FMU. One and two-year-old needles are collected from the upper third of ten trees at each site and taken back to the lab for microscopic examination of pseudothecia density. Three hundred stomata on each of ten needles from each foliage cohort are examined for pseudothecia occurrence (disease severity).

## Results and Discussion

Early aerial surveys for SNC in Washington showed what appeared to be a staggering increase in disease from 1998-2000 (Table 1). Review of the survey data during this time period revealed a record of unstable weather over western Washington throughout the survey window. Disruptions to the aerial survey caused by inclement weather frequently resulted in the survey continuing beyond bud break. At bud break, new foliage casts a chlorotic hue over the forest, adding a layer of ambiguity to the aerial signature. Similarly, the prevalence of western hemlock in coastal forests, prolific cone crops, and other anomalies all contribute to confound disease detection via aerial survey.

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[1] *Department of Natural Resources, Olympia, Washington*

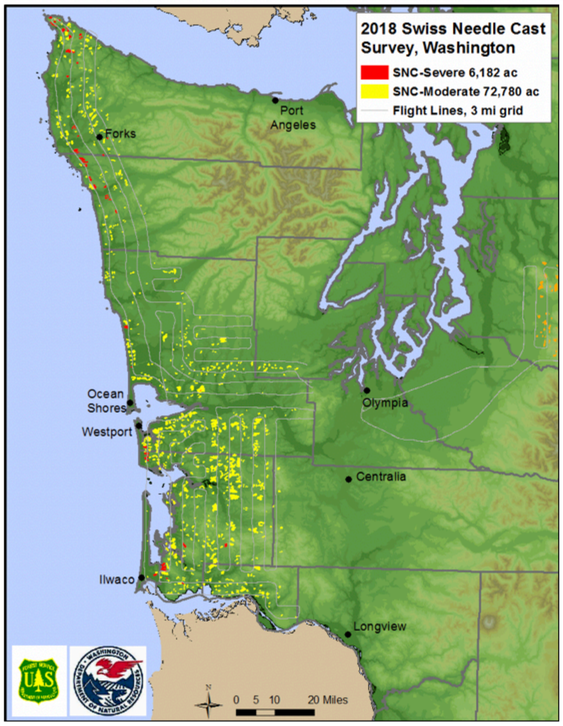

**Figure 1:** *Washington 2018 Swiss needle cast aerial survey map.*

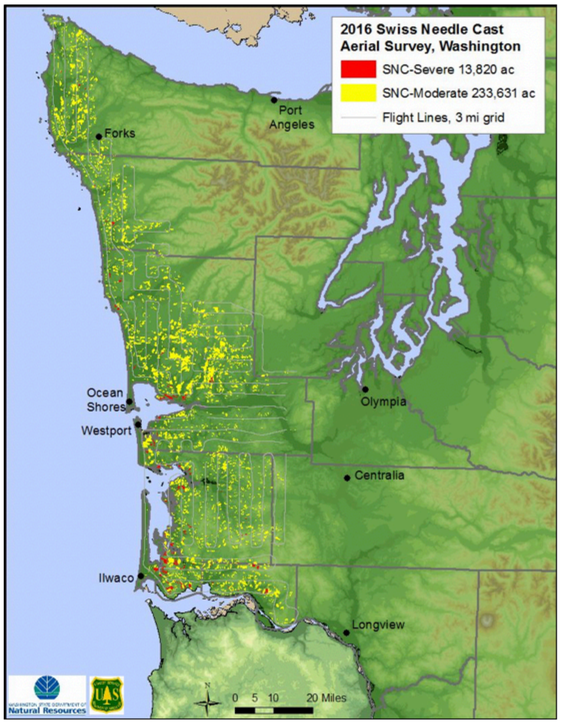

**Figure 2:** *Washington 2016 Swiss needle cast aerial survey map.*

More recent aerial surveys have shown an equally dramatic reduction in acres symptomatic for Swiss needle cast disease (Table 1). In 2018, aerial surveyors flew and made observations on 2.7 million acres of forest land in coastal Washington and mapped 79,000 of Douglas-fir with obvious symptoms of SNC (Figure 1). This is a 68% decrease from the 248,000 acres mapped during the 2016 SNC aerial survey (Figure 2).

The percent of acres surveyed in 2018 symptomatic for SNC is not markedly different from the percent of surveyed acres detected in 1998 (Figure 1). The cause(s) of the decrease in mapped acres from 2015 to 2018 remains uncertain, but may be due to a combination of environmental factors influencing infections patterns and foliar retention, in addition to site and soil characteristics affecting water retention and soil nutrition across sites. Alternatively, it may be the result of aerial surveyors exercising discretion over when to survey and what constitutes an unambiguous signature.

**Table 1:** *Acres of forest symptomatic for Swiss needle cast detected via aerial survey.*

| Year of Survey | Symptomatic Acres | Total Acres Surveyed (%) |
|---:|---:|---:|
| 2018 | 79,000 | 3 |
| 2016 | 248,000 | 10 |
| 2015 | 351,000 | 14 |
| 2012 | 228,000 | 8.5 |
| 2000 | 410,000 | 21 |
| 1999 | 200,000 | 18.2 |
| 1998 | 44,500 | 2.6 |

The objective of the ground survey was to monitor changes in incidence and severity of SNC disease over time. The severity of SNC was measured by counting the number of stomata occluded by fungal pseudothecia on two-year old Douglas-fir needles. The average percentage of occluded stomata across all sites remained relatively stable and varied from 16% to 21% between 2011 and 2018 (Figure 3).

In 2018, needle retention varied across the survey area, ranging from 1.4 to 3.1 years, with trees holding on to a little more than two years of foliage (2.3) across all sixty-three ground survey sites (Figure 4). Many factors, other than disease, are well known to influence needle life span between and within species (Reich et al. 1995). Patterns observed between species can be quite complex and relate to many factors like crown and needle morphology, shade tolerance, and temperature regime and other aspects of the physical environment to which they are adapted. Nevertheless, foliage retention does provide a severity rating that can be operationally implemented in stands ≤40–50 years of age, and it has consistently served as a reliable index of growth loss in Douglas-fir plantations when accompanied by appropriate covariates (Maguire et al. 2002). In spite of the large variability in symptomatic acres detected via aerial survey over the years, disease severity, as measured by pseudothecia occurrence and foliage retention, has remained relatively stable.

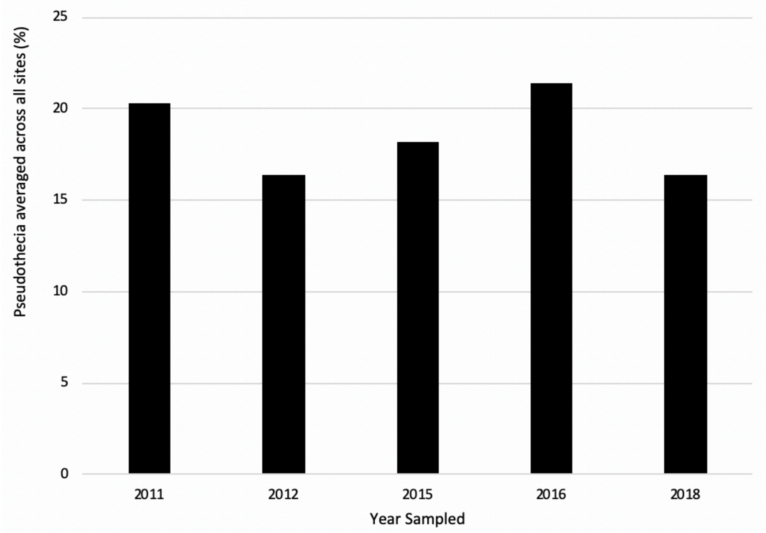

***Figure 3:*** *Average pseudothecia counts/needle from two-year-old Douglas-fir needle cohorts (by year).*

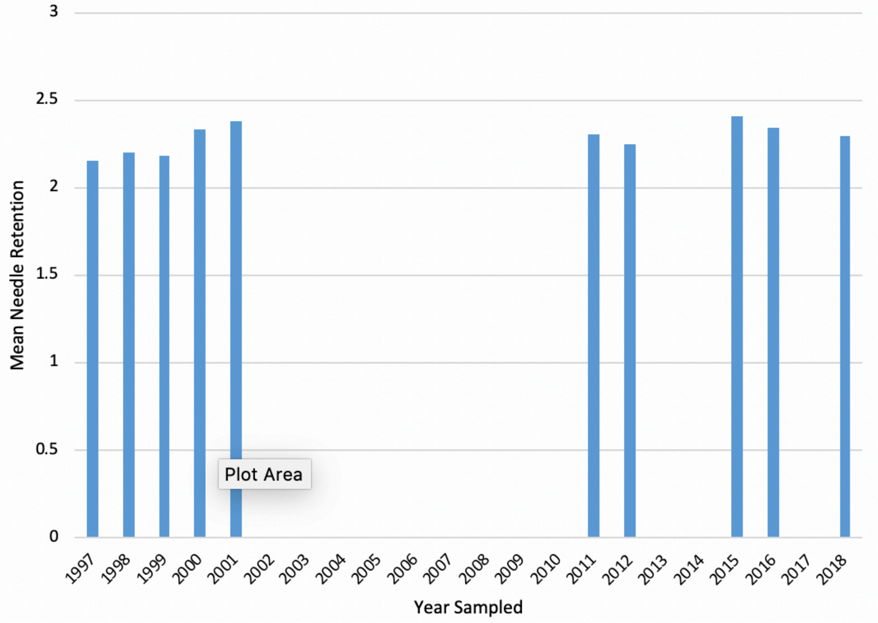

***Figure 4:*** *Needle retention of Douglas-fir from coastal forests infested with Swiss needle cast.*

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## Swiss Needle Cast Monitoring Network – British Columbia

**Stefan Zeglen[1] (presented by Harry Kope[1])**

Swiss needle cast (SNC) has only recently been investigated in coastal British Columbia (BC) as a cause of defoliation of Douglas-fir. Signs and symptoms of the disease have occurred in the Fraser Valley, where plantation aged (10-15 years) Douglas-fir trees showed needle loss to varying degrees. Since there is a paucity of past and useful information on previous outbreaks or occurrences, a new program on SNC has been set up to better understand the conditions for disease occurrence and disease management.

A BC SNC monitoring program was begun in 2017 and as of 2019, 43 monitoring stations have been set up. Weather stations are included at 14 of the monitoring plots. Of the 43 plots, 21 are placed in the very dry biogeoclimatic zones (BEC), 15 in the dry BEC and 7 in the wet BEC (less suitable for Douglas-fir growth). The sampling for branches and needles follows the protocol developed by the SNC Cooperative. In addition to assessment for needle retention and stomatal occlusion, the plots are also used to provide information on soil and foliar analysis and foliar collections for molecular analysis of the pathogen. Weather stations are set up to collect information every half hour on air temperature, barometric pressure, solar irradiance, soil moisture and temperature, relative humidity, vapor pressure deficit, and leaf wetness.

It is anticipated that with the foliage assessments and weather data it might be possible to determine site or seasonal factors that affect the severity of SNC in BC coastal plantations.

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[1] *British Columbia Ministry of Forests, Lands and Natural Resource Operations*

# SEVERITY OF SWISS NEEDLE CAST IN YOUNG AND MATURE DOUGLAS-FIR FORESTS IN WESTERN OREGON, USA*

**Yung-Hsiang (Sky) Lan**[1]

Epidemiology of *Nothophaeocryptopus gaeumannii* is generally well known in plantation trees, but the dynamics of Swiss needle cast (SNC) in older trees is poorly understood. In this study, we compared SNC severity, disease incidence, needle retention, and foliar nitrogen in tree crowns of mature and old-growth forests and nearby young forests at three locations in the Oregon Coast Range and four locations in the western Cascade Mountains of Oregon. At Cascade Head, Klickitat Mountain, Woods Creek, Moose Mountain, Falls Creek, Soapgrass Mountain, and Toad Creek, three old trees and three young trees were sampled at each site (in total 21 mature trees and 21 young trees were sampled across 7 sites). Foliage samples were collected from the upper, middle, and lower crown. Swiss needle cast severity, disease incidence, needle retention, and foliar nitrogen were measured using the SNCC lab protocol. Multivariate ANOVA was used for statistical analysis. Needle wetness sensors were deployed at five sites.

Disease severity on 2-year old needles was greater in younger forests than older forests at all sites. Retention of the 4-year needle cohorts were greater in older than younger trees. Pseudothecia incidence was highest on 2-year-old needles in young trees and on 3-, 4-, and 5- year-old needles in mature trees. Foliar nitrogen concentration differed among years, canopy positions, and sites, but was not different between mature and young trees. Leaf wetness differences were not consistent between young and old tree crowns. Leaf wetness and foliar N were hypothesized to play a controlling role in SNC severity difference between mature and young trees, but while we know these factors are important, they did not explain why disease severity was different.

From this study, we confirmed and provided the quantitative data that young trees have more SNC severity than the adjacent old trees, although we had a small sample size (seven sites). We do not know why SNC severity is different on mature and young trees, but our data suggests that foliage nitrogen content and leaf wetness are not the reason. We also found that disease incidence is different on the needle age class between young trees and mature trees. We suggest that 3- or 4-year-old needles may be better to use to examine SNC on mature trees instead of 2-year-old needles.

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*Published in Lan et al. (2019) *Forest Ecology and Management* 442: p79-95. https://doi.org/10.1016/j.foreco.2019.03.063.

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# DETECTION AND CHARACTERIZATION OF THREE *NOTHOPHAEOCRYPTOPUS GAEUMANNII* LINEAGES OF THE PACIFIC NORTHWEST

**Nicolas Feau[1], Joey Tanney[2], Yin Xue[1], and Richard C. Hamelin[1]**

## Summary

Swiss needle cast (SNC) is a foliar disease of Douglas-fir (*Pseudotsuga menziesii*) caused by the Ascomycete fungus, *Nothophaeocryptopus gaeumanni*. In the Pacific Northwest, SNC has caused an increasingly severe epidemic over the past two decades mostly due to changes in forest management and climate conditions. The SNC disease usually occurs at low elevation in close proximity to the Pacific coast; however, areas of severe impact are projected to increase in the next decades, with northern expansion on Vancouver Island and the Lower Mainland in British Columbia, Canada. Studying the relationship between climate change and adaptability of Douglas-fir and SNC should help to identify the drivers of the current SNC epidemics and the future risks of expansion and epidemics of this disease. Previous population genetic studies of *N. gaeumanni* used microsatellite markers to identify two genetic lineages (designated Lineage 1 and Lineage 2) that may constitute different phylogenetic species (Winton et al. 2006, Bennett & Stone 2016). We sequenced the genome of a worldwide collection of 168 SNC strains, including 136 individuals collected along a latitudinal gradient in the Pacific Northwest (from southern Oregon to Alaska). Our study of 134,403 single-nucleotide polymorphisms (SNPs) revealed that, in additional to Lineage 1 and 2, Lineage 1 can be further divided into 1c ("coastal") and 1i ("interior") (Figure 1A). Distribution of Lineage 1c and 1i follows a coastal/interior split and is consistent with speciation with the host (Coastal versus Interior Douglas fir) and/or climate (Figure 1B). Evolutionary and demographic history modeling suggested a recent split (<20,000 years), but with continued gene migration between Lineage 1c and 1i whereas Lineage 2 diverged from the two other lineages about 140,000 years ago and is genetically isolated. Lineage 1c and Lineage 2 overlap in range (Figure 1B), and we are currently investigating their fine-scale distribution to identify ecological factors that could explain their differentiation. To do so, we developed a multiplex real-time PCR assay which accurately discriminates the lineages from a single pseudothecium. Using this assay, we are examining the presence and distribution of lineages 1c and 2 in 36 Douglas fir stands in coastal British Columbia where we collected needles of different age and different location on the trees. Expanding our knowledge on the genomics, distribution, and ecology of SNC lineages will improve our ability to predict risk associated with future outbreaks of the disease and help develop efficient breeding strategies and transfer protocols of Douglas-fir material in climatically and phytosanitary suitable regions.

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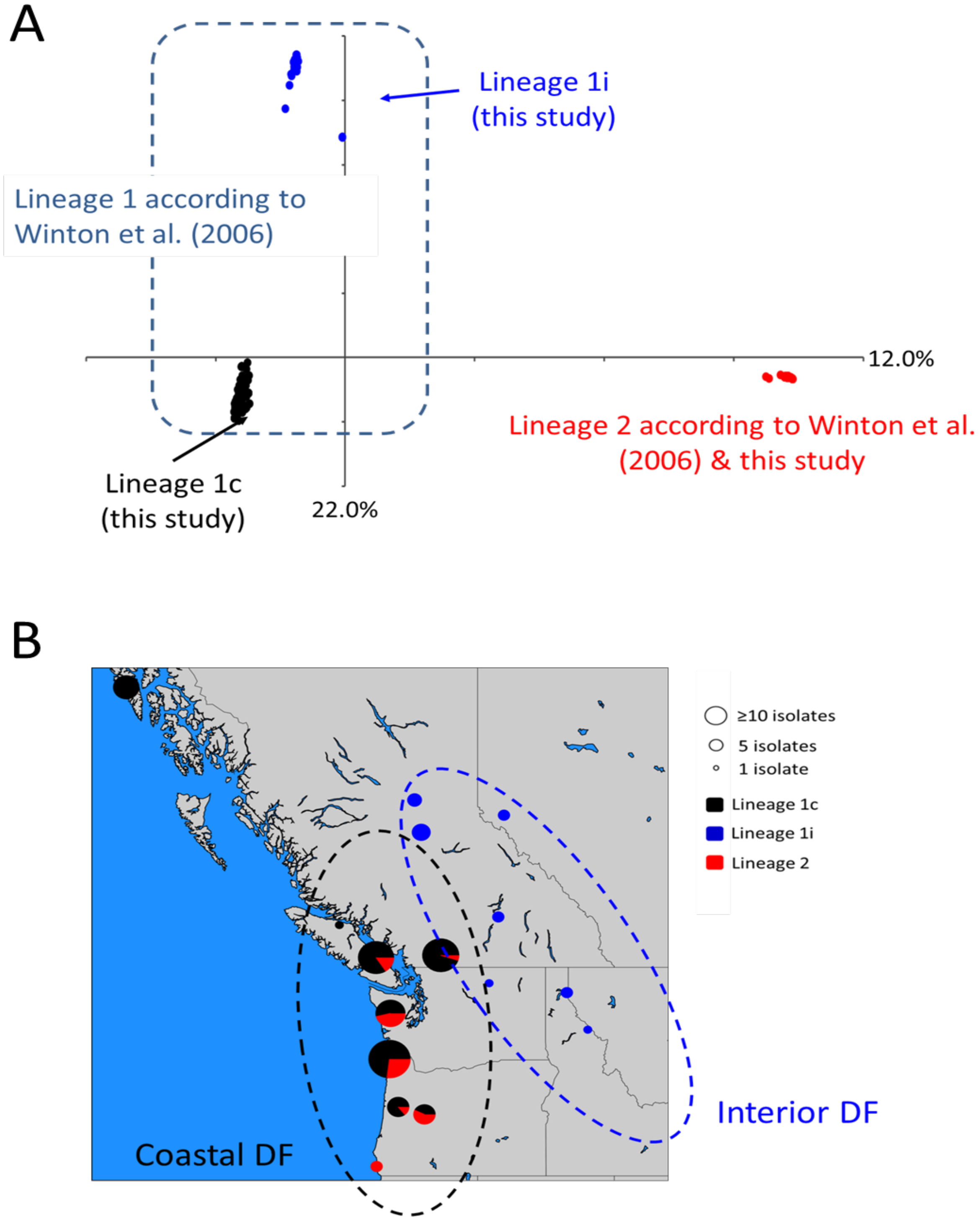

***Figure 1:*** *Genetic structure of the global population of the Swiss needle cast.* ***A.*** *Principal component analysis based on 134,403 SNPs; the three putative SNC lineages are indicated with different colors.* ***B.*** *Geographical distribution of the genomes sequenced.*

# SWISS NEEDLE CAST IN BRITISH COLUMBIA'S COASTAL DOUGLAS-FIR BREEDING POPULATION

**David Montwé[1], Jürgen Ehlting[1], and Michael Stoehr[2]**

With changing climates, there is increasing concern in western Canada regarding the impact of Swiss Needle Cast, a native fungus causing premature needle loss in Douglas-fir *Pseudotsuga menziesii*. Low needle retention is associated with growth losses up to 30-50% in this otherwise productive and valuable timber tree species. While historically of lower concern, epidemics in the southern range of coastal Douglas-fir as well as modelling suggest higher disease risk in the coming decades in coastal British Columbia. To address this risk, we: 1) screen an advanced-generation breeding population for variation in disease resistance or tolerance, 2) evaluate heritability of tolerance, and 3) understand the molecular basis of disease response to facilitate marker-assisted selection in subsequent breeding cycles using RNA-seq. We evaluated signs of the pathogen itself (fungal fruiting bodies occluding stomata) as well as symptom severity (needle retention) on two climatically different sites over three years, allowing us to disentangle the effects of genotype and environment. There are differences in needle retention and fruiting body counts among families, sites, and years. In addition to this variability, tolerance to the disease appears to be under moderate genetic control. Preliminary results are presented, and implications are discussed.

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# PANEL: SPECIAL PAPERS (MODERATOR: KELLY BURNS)

# THE NATIONAL WHITEBARK PINE RESTORATION PLAN: A MULTI-AGENCY COLLABORATIVE EFFORT TO RESCUE A HIGH ELEVATION KEYSTONE AND FOUNDATION SPECIES

**Diana F. Tomback,[1] Eric Sprague,[2] and David Gwaze[3]**

Whitebark pine (*Pinus albicaulis* Engelm.) is declining rapidly from an unprecedented combination of threats—especially the exotic pathogen *Cronartium ribicola* but also large-scale outbreaks of mountain pine beetles (*Dendroctonus ponderosae*), altered fire regimes, and climate change (Tomback & Achuff 2010, Schwandt et al. 2010). Although restricted to subalpine and treeline elevations, whitebark pine occurs in multiple forest cover types in the U.S. and across > 4 million ha, throughout the Sierra Nevada and Cascades and northern Rocky Mountains (McCaughey & Schmidt 2001, Goeking & Izlar 2018) (Figure 1). Whitebark pine grows on productive sites as an early seral species in successional subalpine mixed forest communities, as a self-replacing and dominant climax species on exposed sites with poor soils, and as an important component of many treeline communities (Tomback & Achuff 2010, Tomback et al. 2016). Its distribution, population biology, and ecology are influenced by the seed caching behavior of Clark's nutcracker (*Nucifraga columbiana*), its obligate seed disperser (e.g., Tomback 2005).

Whitebark pine is known for its ecological role as a keystone species, fostering community diversity by providing wildlife large, nutritious seeds, as well as protective habitat and nest sites at the highest forest elevations (e.g., Tomback & Achuff 2010, Tomback et al. 2011). It is also recognized as a foundation species because it rapidly colonizes after disturbance, facilitating the establishment of other species and redistributing and retaining snowpack at the highest elevations (Ellison et al. 2005, Tomback et al. 2016, DeGrassi et al. 2019).

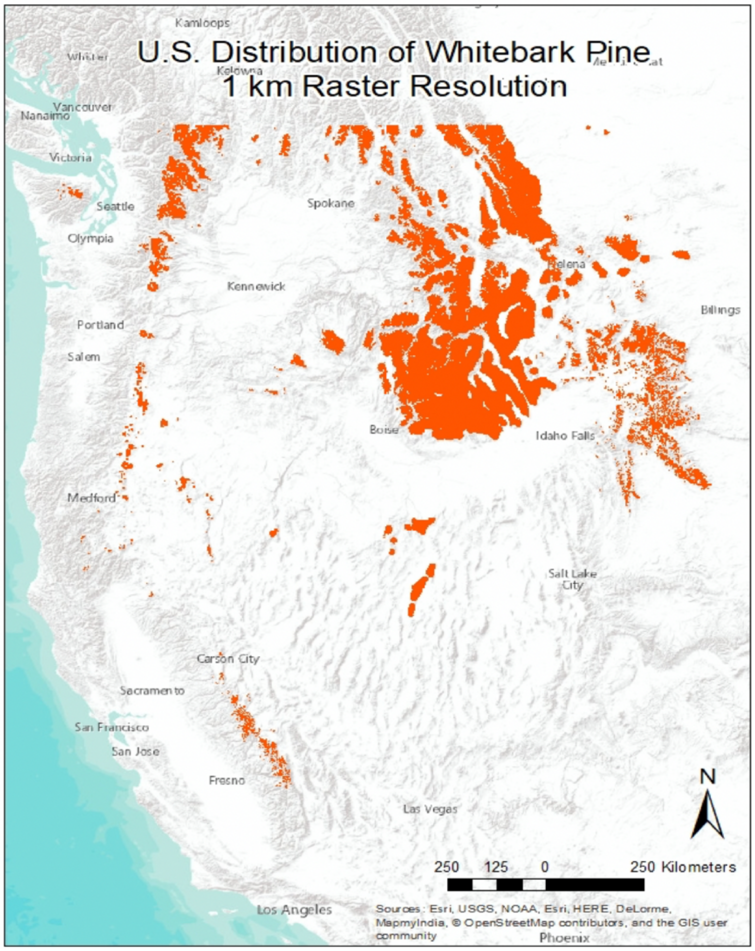

***Figure 1:*** *Refined distribution map at 1 km resolution for whitebark pine (*Pinus albicaulis*)—product of Data Call 1 of the National Whitebark Pine Restoration Plan. Map layers and supporting information are available at https:// whitebarkfound.org/our-work/national-whitebark-pine-restoration-plan/data-call-1/.*

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In 2011, the U.S. Fish & Wildlife Service designated whitebark pine a candidate species for potential listing under the Endangered Species Act (U.S. Fish & Wildlife Service 2011). Meanwhile in 2012, whitebark pine was listed as Endangered in Canada under the Species at Risk Act (Government of Canada 2012). After various delays, the U.S. Fish & Wildlife Service recently completed an evaluation of the pine, and a listing decision is now expected in 2020. If listed, whitebark pine would be the most widely distributed forest tree under ESA protection, with more than 88% of its distribution on federally managed lands.

Given the magnitude of whitebark pine's range and the extent and severity of whitebark pine losses (e.g., Goeking & Izlar 2018), management intervention in the form of restoration poses logistical challenges and budgetary challenges. In 2016, the Whitebark Pine Ecosystem Foundation and American Forests proposed a collaboration with the U.S. Forest Service Washington Office to develop the National Whitebark Pine Restoration Plan (NWPRP). The Whitebark Pine Ecosystem Foundation is lead on the development phase for the scientific plan, while American Forests will assume the key role in fundraising for plan implementation.

## Restoration Practices

Restoration tools and protocols for whitebark pine have been developed and implemented for small-scale whitebark pine projects for more than two decades (Tomback et al. 2001, Sniezko et al. 2011, Keane et al. 2012). *Cronartium ribicola*, the pathogen that causes the disease white pine blister rust, is the primary threat to whitebark pine, reducing cone production and potentially extirpating populations. A small proportion of trees throughout whitebark pine's range have resistant phenotypes, which can be determined through a multi-step screening process. Once individuals with genetic resistance are identified, managers may develop plans to protect these trees from mountain pine beetles and fire, so their seeds can be collected for operational seedling production over time.

The primary restoration strategy for whitebark pine is to accelerate natural selection by planting progeny potentially resistant to blister rust in open seedbeds, such as recent subalpine burns or where overstory has been killed by beetle outbreaks. For areas within whitebark pine's range with low blister rust incidence, resilience and resistance can be developed before infection levels increase substantially, again through planting of resistant seedlings but also by providing regeneration opportunities (e.g., Schoettle & Sniezko 2007). Other important restoration techniques include silvicultural thinning to reduce competition in successional stands and prescribed burning to open stands more effectively and reset succession (Keane et al. 2012). In addition, whitebark pine restoration can be directed to areas and topography that will be slower to experience climate warming. We expect that agencies will use appropriate management practices in relation to whitebark pine restoration, as well as a monitoring and adaptive management strategy to examine the effectiveness of restoration work.

## The National Whitebark Pine Restoration Plan

The National Whitebark Pine Restoration Plan (NWPRP) is a collaborative effort among all federal agencies, tribal governments, and state agencies that have management jurisdiction for whitebark pine. Agency management responsibilities as percent of whitebark pine's range are as follows: U. S. Forest Service—74%, National Park Service—10%, Bureau of Land Management—4%, and, state, private, and tribal lands—12%. About 29% of whitebark pine's range occurs in Wilderness Areas and designated wilderness across agencies (U. S. Fish & Wildlife Service, unpublished data). Guidance for steps and processes for the NWPRP has been provided by an NWPRP Liaison Committee with representatives from all agencies, including tribal government.

As stated in the NWPRP, the general goal of whitebark pine conservation and restoration is to develop and sustain healthy and resilient whitebark pine communities in the face of current and future challenges. The purpose, however, of the NWPRP is to focus scarce resources on core areas nominated for restoration to ensure that a proportion of whitebark pine's distribution develops or retains healthy and resilient whitebark pine populations. The restored whitebark pine populations within agency and tribal-designated core areas will serve as the "dissemination centers," that is as source populations, for the spread of whitebark pine and especially genotypes resistant to white pine blister rust, to less healthy or untreated areas over time through the seed dispersal services of Clark's nutcrackers. Trees within nominated core areas are expected to maintain genetic diversity and to evolve high levels of blister rust resistance through restoration projects such as planting resistant seedlings and through enhanced natural regeneration efforts. By focusing resources on selected portions of whitebark pine's distribution, we have a greater chance of creating healthy and resilient whitebark pine populations that maintain functional communities on the landscape, including provision of ecosystem services.

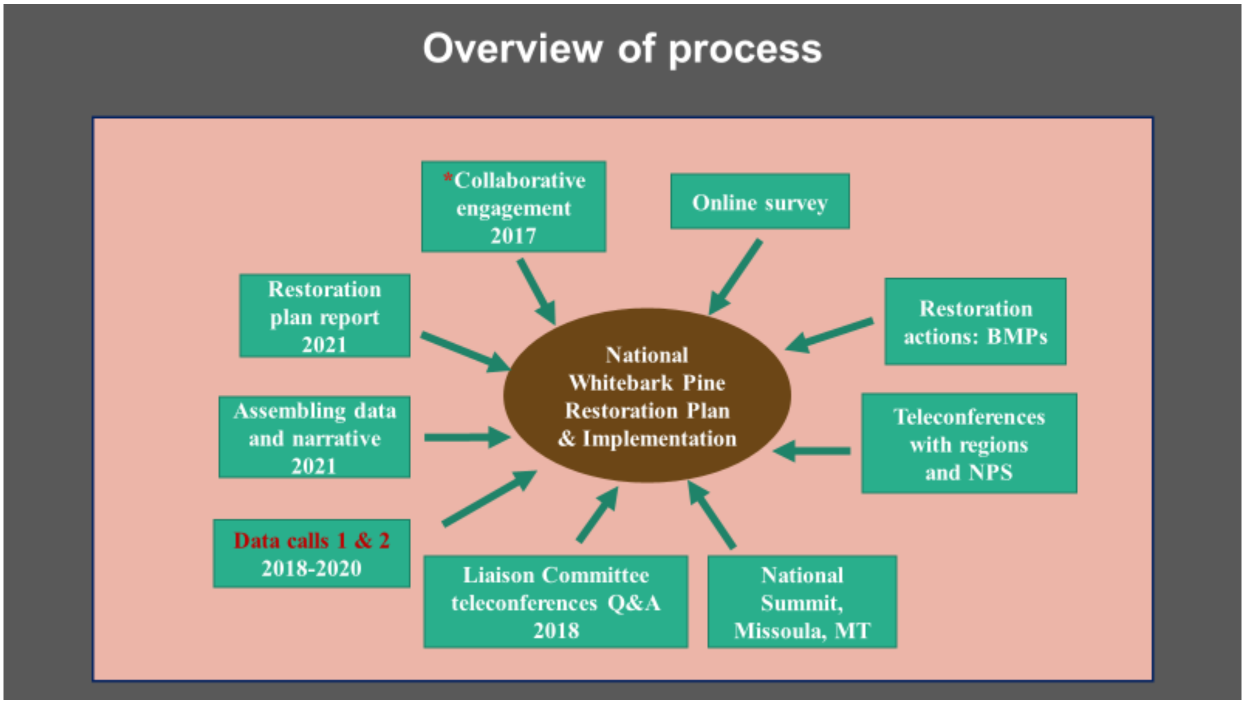
The NWPRP relies on the nomination by all federal, tribal, and state agencies of selected areas within their jurisdiction for restoration treatments, using a combination of biological criteria that reflect the U.S. Fish & Wildlife Service's Species Status Assessment Framework (U.S. Fish & Wildlife Service 2016) and agency-specific criteria. Core area nominations in the form of polygons are expected to represent roughly 20 to 30% of the whitebark pine distribution within an agency or tribe's jurisdiction; and, in total, the core areas nominated collectively should represent 20 to 30% of whitebark pine's U.S. distribution. Agencies have the latitude to decide whether each management unit, e.g., national park, state, or national forest, should nominate 20-30% of their whitebark pine or whether the combined total for each regional jurisdiction should comprise 20-30%. More detailed information guiding the process of nominating core areas is available at https://whitebarkfound.org/our-work/national-whitebark-pine-restoration-plan/.

We have encouraged agencies and tribes with contiguous whitebark pine communities to collaborate on core area decisions. The core areas, in turn should be recognized within each agency or tribe as having high priority for implementing whitebark pine restoration actions. The timeline for completing restoration within agency core areas, which includes verification of acceptable levels of success, is 10 to 15 years after the restoration plan is finalized (2030-2035), although faster tracking is highly recommended. Core area nominations do not preclude restoration actions in non-core areas of distribution, although they should be considered lower priority. At this point in time, we are in the middle of Data Call 2A, which is the request for nominated core areas.

## Products of the Plan and Next Steps

The draft of the complete NWPRP is projected to be available late in 2021. The components to the restoration plan include: the nominated core areas (polygons) for restoration; the criteria used to select core areas; the proposed restoration action(s) within each nominated polygon; the implementation costs within each polygon; and a monitoring and adaptive management sub-strategy for insuring the effectiveness of restoration. The steps leading up to development of the plan and the data calls are outlined in Figure 2.

The NWPRP products are anticipated to include a U.S. Forest Service General Technical Report with maps and a detailed narrative, a best management practices report, and a summary of the plan for citizen outreach and fund-raising purposes. The completion of the report signals the implementation phase of restoration, which will require fund-raising through collaboration, partnerships, and outreach. In other words, the completion of the plan is the beginning of the real work.



Overview of process

National Whitebark Pine Restoration Plan & Implementation

*Collaborative engagement 2017

Online survey

Restoration actions: BMPs

Teleconferences with regions and NPS

National Summit, Missoula, MT

Liaison Committee teleconferences Q&A 2018

Data calls 1 & 2 2018-2020

Assembling data and narrative 2021

Restoration plan report 2021

***Figure 2:*** *This figure summarizes the sequence of activities involving organization and development of process in the National Whitebark Pine Restoration Plan. Collaborative engagement began with a letter from Leslie Weldon, Deputy Chief of the National Forest System, announcing the roll-out of the plan in May 2017. This event was followed by a series of outreach meetings, including a technical workshop synthesizing best management practices (BMPs) for whitebark pine, followed by the National Whitebark Pine Summit in Missoula, MT, in November 2017.*

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EXIT

# *PHYTOPHTHORA* PATHOGENS THREATEN FORESTS AND RESTORATION SUCCESS

**Susan J. Frankel**[1]

First recognized in the mid-1990s, the sudden oak death pathogen, *Phytophthora ramorum,* continues to defy control or management. An estimated 43 million *Notholithocarpus densiflorus* (tanoak) and *Quercus agrifolia* (coast live oak) trees have been killed by *P. ramorum* in 15 coastal California counties and in southwest Oregon (Curry County) (Cobb et al., *submitted*). The pathogen continues to spread, primarily via windblown rain, or inadvertently, on shipments of infected ornamental nursery stock (i.e. *Rhododendron*). In the U.K. and Ireland, the pathogen is damaging *Larix kaempferi* (Japanese larch) in plantations (Grünwald et al. 2019). Here, I provide a capsule summary of current issues related to *P. ramorum* as well as concerns over the introduction of other *Phytophthora* species into California restoration areas. For more information on sudden oak death and *P. ramorum*, see www.suddenoakdeath.org.

**New *P. ramorum* hosts – rare plants**. Remarkably, from 2015 to 2017, *P. ramorum* was first recognized on 11 rare, threatened, or endangered *Arctostaphylos* species (manzanitas), in wildlands and botanic gardens (Frankel et al., *in press*). Symptoms appear as leaf spots, dieback, or complete plant mortality. The detections occurred in areas known to be infested with *P*. *ramorum* for several decades, but symptoms on manzanita species had not previously been reported. The infestations threaten species already designated as rare or endangered and degrade high-value habitats. These findings illustrate how an exotic, invasive pathogen can become established on common hosts but then over time spread to rare species, damaging plant collections and conservation plantings.

**Inadvertent shipments to the Midwest and East of *P. ramorum* infected nursery stock (*Rhododendron*, *Syringa* (lilac) and *Kalmia*).** Despite the federal quarantine, in July 2019, USDA Animal and Plant Health Inspection Service (APHIS) reported the 'Detection of *Phytophthora ramorum*-Infected Plants in Commerce' confirming that plants in Indiana nurseries tested positive for *P. ramorum*. The infected plants, part of large shipments that originated from nurseries in Washington State and Canada, were sent to 28 states throughout the Midwestern and Eastern U.S. (COMTF 2019a). The shipments harbored the NA2 *P. ramorum* clonal lineage, an uncommon clone not known to be present on forest vegetation in the U.S. (COMTF 2019b). As of December 2019, APHIS and state officials continue trace-back and trace-forward investigations in an effort to track down and destroy infected plants.

***Phytophthora* detections in native plant nurseries and restoration sites.** Coming nearly two decades after the start of the sudden oak death pathogen outbreak, a number of first U.S. detections of *Phytophthora* species are heightening concerns that the nursery to wildland *Phytophthora* pathway may once again, inadvertently, initiate an uncontrollable, wildland plant disease epidemic. In California, in 2012, a first-in-the-U.S. detection of *P. tentaculata* on sticky monkey flower (*Diplacus aurantiacus* subsp. *aurantiacus*) was reported on container plants in a native plant nursery in Monterey County (Rooney-Latham & Blomquist 2014) with subsequent detections on several native species in nurseries and reforestation sites in several California counties (Rooney-Latham et al. 2015). Further investigations detected another first-in-the-U.S. record, *P. quercina* on a planted *Quercus lobata* (valley oak) in a restoration area in San Jose (Santa Clara County). *Phytophthora quercina* is considered potentially highly damaging to U.S. environmental and economic interests (Swiecki et al. 2018). In 2014-16, a voluntary survey

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of California native plant nurseries and restoration areas identified well over 25 *Phytophthora* taxa and at least 70 new *Phytophthora* native plant associations in nurseries and restoration sites (Rooney-Latham et al. 2019). These soilborne plant pathogens detected as moving from native plant nurseries to restoration sites on planting stock may potentially undermine the very purpose of restoration projects, degrading rather than enhancing habitat. To protect sensitive habitats and restoration sites against the risk of *Phytophthora* and other plant pathogen introductions the Phytophthoras in Native Habitats Work Group was formed (Frankel et al. 2018). For more information, see www.calphytos.org.

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# EMERGENCE OF WHITE PINE NEEDLE DAMAGE IN THE NORTHEASTERN UNITED STATES IS ASSOCIATED WITH CHANGES IN PATHOGEN PRESSURE IN RESPONSE TO CLIMATE CHANGE

**Stephen Wyka[1], Cheryl Smith[2], Barrett Rock[3], Isabel Munck[4], and Kirk Broders[5]**

Since 2010, eastern white pines (*Pinus strobus*) across the northeastern United States have been prematurely defoliating due to a disease complex known as White Pine Needle Damage (WPND). Previous research on WPND indicates that this outbreak is primarily attributed to the causal agent of brown spot needle blight, *Lecanosticta acicola,* along with two other known *P. strobus* needle cast pathogens, *Bifusella linearis* and *Lophophacidium dooksii.* The current outbreak is thought to have arisen from several consecutive years of increased temperature and precipitation in the region. Current research, however, is lacking in both sampling distribution, the basic epidemiology of the primary causal agents, and the specific climatic factors that affect the development and spread of this outbreak. Furthermore, recent re-sampling of diseased *P. strobus* needles within this region has revealed a high isolation frequency of a putative new species closely related to *Septorioides pini-thunbergii*, a species associated with needle cast of *Pinus thunbergii* in Japan, which was initially not known to be associated with WPND. This research sought to describe and classify the new species associated with WPND, expand field-based sampling of WPND to construct a more detailed distribution map, utilize long-term WPND monitoring plots and land-based weather stations to create a climatic model to predict the severity of defoliation events in the next year, and establish a field-based spore trapping experiment to determine how climatic factors affect the development and dispersal of *L. acicola* spores. Results demonstrate the widespread occurrence and establishment of a new family (Septorioideaceae) and a new species, *Septorioides strobus*, as well as its association with the current WPND outbreak. Continued sampling provided further support for the disease complex theory that *L. acicoa*, *B. linearis*, *L. dooksii*, and *S. strobus* were ubiquitous across the region and neither an individual species nor a specific combination of species had a dominating presence in particular states or regions but were generally found in forest stands that comprised >50% eastern white pine by basal area. Additionally, regional weather data confirmed the trend of increasing temperature and precipitation observed in this region, with the previous year's May, June, and July rainfall being the best predictor of the following year's defoliation event, while the current year's relative humidity was responsible for spore development and increased inoculum loads.

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# STRUGGLING TO IDENTIFY CAUSAL AGENTS OF NEW DISEASES: DISTINGUISHING ENDOPHYTES FROM LATENT PATHOGENS

**Gerard Adams[1], Loretta Winton[2], and Robin Mulvey[3]**

This discussion was inspired by a comment concerning diagnosis of canker diseases on trees: Dr. Gary Chastagner asked, "*How do you complete Koch's Postulates with endophytes?"* This was a cogent question and a pressing issue because many new and devastating canker diseases have recently demanded our attention and isolation of the causal agents have been problematic. The logic behind this question is that it is assumed an endophyte will not cause disease symptoms when inoculated onto the host species from which it was isolated: Why bother? And here we need to clarify that the discussion is about reproducing the disease symptoms, not recording colonization of host tissue.

In the past 10 years my research program has involved just that, inoculating "endophytes" onto the host trees and attempting to complete Koch's Postulates to verify pathogenicity. And the results of these efforts have been unpredictable, unexpected, and revealing. Essentially, canker margins have been yielding several fungi and these fungi are not readily identifiable because they have not previously been considered pathogens. Identification of fungi has shifted paradigms and is today based on DNA sequence and finding sequence homology in the NCBI Genbank database, followed by cultural and microscopic morphological study, rather than the historical morphological identification paradigm. The worldwide database, at this time, is relatively depauperate of tree inhabiting fungi other than from extensive study of endophytes, primarily by Dr. Elizabeth (Betsy) Arnold and her students at the Robert L. Gilbertson (Forest) Mycology herbarium at University of Arizona.

The purpose of this presentation is to emphasize 1) a need for pathologists to initiate studies of Koch's Postulates using fungi isolated from diseased tissues that are generally considered endophytes based on sparse literature and reports of habitat in NCBI Genbank databases, and 2) a need to publish lists of endophytes isolated from forest trees at the time of disease diagnosis, while many are not identifiable publishing the DNA sequence of a few phylogenetically-relevant gene sequences will serve to permit all future identifications of unnamed and unknown fungi encountered. Uploading (populating) the NCBI database with such sequences accompanying host, location, habitat and other relevant information from the study, such as publication or report titles, eventually will greatly expand the resources for effective pathology work.

Presented here are the outcomes of such studies on three different hosts suffering undiagnosed canker diseases of significant concern.

New and sudden forest diseases of the decade include four that I have participated in studying: 1) Sudden Aspen Death (of *Populus tremuloides*) in the Rocky Mountains, 2) *Pinus strobus* branch dieback & mortality in Lake States, 3) *Tsuga heterophylla* branch dieback and mortality in Alaska, and 4) Aspen Running Canker in Alaska. Determining the etiology of tree diseases has been outlined as involving three parameters, defining the inciting factor(s), the predisposing factor(s), and the contributing factor(s). Proximate causes kill trees debilitated by predisposing & inciting factors and these are referred to as the contributing factors. The latter include the pathogens and pests causing disease symptoms.

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In the case of Sudden Aspen Death (SAD) endophytic fungi from symptomatic tissues were not included in identification or inoculation studies. Based on work on aspen cankers, we now believe that this needs to be revisited and accomplished with thorough practice. Recent pathology research such as the great multinational effort on understanding SAD reported some identified insect damage and Cytospora canker as minor contributing factors. Might a significant culprit be missing? Are we failing to identify the contributing factors?

White pine branch dieback and mortality in the Lake States initially appeared as a new disease occurring in proximity to Au Sable and Manistee rivers. The symptoms were classic running diffuse cankers showing discolored cambium in the current year and wilting of foliage (flagging) on affected branches followed by branch dieback which in some trees progressed into the trunk causing mortality. The result was death of much of the regeneration planned for replacement of harvested stands. Visible branch dieback, flagging, and some mortality throughout young pine stands. Contributing factors from our studies included adult Spittle bug feeding injury to cambium on the affected branches and the canker. In study of the fungi isolated from the margin of the advancing brown cambium tissue were the "latent pathogen/endophyte" *Diplodia scrobiculata, Therrya pini, Phialocephala fortinii, Phaeomoniella* sp., *Microdiplodia* sp*.,* and *Xylaria* sp. Casual diagnosis likely would have concluded that *Diplodia scrobiculata* was the causal agent, but the incidence of isolation of this fungus was far too scarce to accept that diagnosis. Additionally, inoculation trials on saplings chosen as representative of stressed trees in the location of origin failed to result in disease symptoms. Koch's Postulates were attempted using the six fungi in replicated plots chosen as stressed (high Spittle bug populations along the river banks) and unstressed (sites with no canker or spittle bugs along the river banks). Not surprisingly, none of the fungi caused disease symptoms. Additionally, re-isolation from inoculated branches usually resulted in a different endophytic fungus being cultured than the one used in the inoculation of the branch. This effort is what would be expected and verified the issue raised by Dr. Chastagner's question. However, we did learn what fungi occur in *P. strobus* in the region of the damage. Also, based on frequency of isolation it was discovered that *Therrya pini* was most successful in ramifying through the browning cambium tissues during senescence. This is new information that may aid future pathologists in diagnosis of branch mortality.

Quite the opposite result has followed study of the remarkably similar disease on western hemlock branch dieback and mortality that periodically damages stands across the extent of the Tongass National Forest in Southeast Alaska. This pathogen causes synchronized mortality of lower branches and small trees (Figure 1), and is most notable along forest openings like roads and rivers. Our studies resulted in isolation of many different fungi from the rapidly advancing margins of diffuse branch cankers, including: *Pezicula pruinosa, Pezicula aurantiaca*, *Caliciopsis* sp., *Collophora* sp. (*Coniochaeta* sp.)*, Dermea padi, Discocainia treleasei, Neonectria* sp.*, Ophiostoma piceae, Pestalotiopsis* sp., *Sarea resinae, Sydowia* sp., and *Zalerion arbicola*. In this study over 775 saplings were inoculated with these fungi and others in effort to complete Koch's Postulates. In this study spanning 4-5 years, several of the fungi caused reproducible canker symptoms like the field observations (Figure 2). The color of some cankers matched the lesions caused by the pathogen, while others did not. For example, *Zalerion arbicola* caused a much darker brown lesion compared to the light reddish-brown lesion of the causal pathogen. The fungi inoculated onto a branch were generally re-isolated following canker measurements. Proximate cankers with fruiting structures occurring in the affected forest stands were those caused by *Discocainia treleasei*. *Discocainia treleasei* also was highly virulent in inoculation trials but it was rarely isolated from the naturally diseased trees. *Neonectria* sp., *Ophiostoma piceae, Dermea padi* and the *Pezicula* spp. were all found capable of reproducing the canker symptoms. In fact, *Neonectria* sp. and *Ophiostoma piceae* were isolated from inoculated saplings where we noticed two different cankers based on cambium color, and the two fungi

***Figure 1:** Crown symptoms of the branch dieback and mortality disease of* Tsuga heterophylla*.*

were causing a faster-advancing canker of lighter color than the inoculated fungus. With confounding results from Koch's Postulate studies, we employed next generation sequencing using barcoding genes to identify every fungus in the cankered margins to verify that we had not missed the causal agent. In fact, we discovered that the most prominent fungal DNA amplified in the tissues had only been isolated once, this fungus was tentatively identified by BlastN as a *Chalara* sp. related most-closely to *Xenopolyscytalum* sp. These methods also detected consistent presence and relative abundance of *Pezicula* sp. (*Pezicula livida* group) in cankered tissue. We had also missed an *Allantophomopsis* and a *Cladophialophora* species. This spurred us to complete Koch's Postulates with cultures of these species in the last season of study. The results of the exhaustive study are conclusive; based on the frequency of isolation from canker margins and reproducible virulence in replicated inoculation trials that the actual pathogen in the natural cankers is a species of *Pezicula*. This is despite the fact that it is not the most abundant fungus in the diseased tissues (which was the non-pathogenic endophyte *Xenopolyscytalum*), nor the virulent proximate *Discocainia,* and the rare *Neonectria*. This is a surprising but sound conclusion. The discovery of the contributing factor in this widespread sporadic and periodic disease required exhaustive Koch's Postulate trials and thorough documentation of endophytic fungi. The genus *Pezicula* contains high numbers of species recognized in the literature as "simple" endophytes, many under the anamorph name *Cryptosporiopsis.* However, inoculation studies proved one *Pezicula* was the virulent pathogen. Additionally, considerable new information important to understanding pathogens of western hemlock was gained by pursuing the inclusion of endophytes in Koch's Postulate studies.

***Figure 2:** Cankers resulting from inoculation trials caused by several of the tested fungi. For canker size reference, images are sized to match a uniform metal tag diameter (13mm).*

Aspen Running Canker disease in Alaska is causing extensive and widespread mortality in many age classes of aspen in interior Alaska. We have just succeeded in identifying the causal agent but only after replicated trials pursuing completion of Koch's Postulates. Again, several fungi not known as pathogens in the literature, fungi expected to be endophytes, and fungi often not accurately identified using DNA sequence or morphology, were included in the study (we had already tested for and excluded potential Oomycete pathogens, bacterial pathogens, nematodes, and insect pests). Again, we employed next generation sequencing using barcoding genes to identify every fungus, bacterial species, and other eukaryotic microorganism present in the rapidly advancing margins of the diffuse cankers. The most abundant fungus in the diseased tissues was a common food grade yeast and not the pathogen, but the pathogen itself was also an uncharacterized yeast. Surprising new information in forest pathology resulted from this study due to including endophytic fungi and fungi assumed to be endophytes and having no prior literature involving pathogenicity.

In summary, the tree species appears to influence the results of inoculation trials using endophytes. White pine showed no virulence and uncertain colonization, and unsuccessful re-isolation following inoculation. Whereas, western hemlock showed low, moderate, and high virulence depending on the uncharacterized, presumably endophytic, fungal species tested. Additionally, for western hemlock, all the fungi in the inoculation trials were readily re-isolated following canker measurements. Similarly, the aspen inoculations responded intermediately compared to the white pine versus the western hemlock. But all the efforts that included endophytes and presumed endophytes in Koch's Postulate trials resulted in important and crucial new information in forest pathology which needs to be recorded in NCBI records and elsewhere in publication. We therefore emphasize the need for reasonable recording, populating of GenBank, and testing Koch's Postulates with host-identified endophytic fungi to be routine in current and future studies of forest pathogens.

# PANEL: RUSTS (MODERATOR: NICHOLAS WILHELMI)

# EVALUATING THE PRESENCE AND INTROGRESSION OF THE HYBRID FOREST PATHOGEN *CRONARTIUM X FLEXILI*

**Kiah Allen[1], Nicolas Feau[1], and Richard C. Hamelin[1]**

## Summary

The recent discovery of a hybrid forest fungal pathogen, *Cronartium x flexili*, suggested to have arisen from sexual hybridization between the introduced *C. ribicola* and the native *C. comandrae* rusts, was surprising because the parental species do not share hosts. The white pine blister rust pathogen *C. ribicola* was accidentally introduced in North America 100 years ago and has devastated white pines of the subgenus *Strobus*. The comandra blister rust pathogen *C. comandrae* is native in North America, infecting 2- and 3-needle pines of the subgenus *Pinus*. Although the pathosystems of both parental species are well described, the impact of their hybridization is yet unknown. The purpose of this study was to determine the occurrence, level of hybridization, and introgression of *Cronartium x flexili*. A total of 831 isolates from dikaryotic aecia of *C. ribicola* and *C. comandrae* were collected from thirteen sites across British Columbia and Alberta in Canada and analyzed both morphologically and genetically over two sampling seasons. Molecular biology methods including PCR, qPCR, and genotyping by sequencing (GBS) were used to identify hybrid isolates and assess the level of introgression. The analysis of spore morphology and the results of these genetic and genomic analyses indicated that *C. x flexili* is either no longer prevalent in areas where it was previously found, or else prevalent at such low frequencies that it has evaded detection.

Two previously collected *C. x flexili* samples were examined using a fixed loci analysis and did not clearly indicate whether or not introgression was occurring. Thus, when a hybridization event occurs between the parental *Cronartium* spp., first generation hybrids result but it is unclear whether or not they are fertile. We hypothesized that the absence of hybrids found in the sampling efforts of this study could be due to the ‘hybrid breakdown’ as explained by the Bateson-Dobzhansky-Muller incompatibility model. This would result in low relative fitness of *C. x flexili* compared to the parental species. Variations in local climate factors influencing life cycle parameters of the two parental species may also explain the low abundance of this hybrid.

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# GENETIC RESISTANCE TO WHITE PINE BLISTER RUST IS KEY BUT HOW IT IS MANAGED WILL DETERMINE THE FUTURE OF HIGH ELEVATION FIVE-NEEDLE PINES

**Anna W. Schoettle[1]**

The North American high elevation five-needle white pines (Figure 1) define the forest-alpine ecotone in many mountain systems and provide watershed protection and wildlife habitat. Climate change is projected to be rapid and heightened in these same habitats, highlighting the importance of genetic diversity and adaptive capacity of the species that occupy them (Millar et al. 2007). The five-needle pines are also being challenged be an invasive fungal pathogen, *Cronartium ribicola*, that was introduced to North America in the early 1900s and causes the lethal disease white pine blister rust (WPBR) on five-needle white pines (subgenus *Strobus*) (Figure 2). All the high elevation five-needle white pines of North America are susceptible to *C. ribicola*. The disease spread rapidly through host ranges in the moist forests of the northwest and east and continues to spread, though more slowly, into the drier habitats of the southern Rockies, Great Basin, and southwest. WPBR has killed many five-needle white pines in the north, though wasn't detected in Colorado until the late 1990s and has yet to be found on trees in Utah or most of Great Basin (see map in Jacobi et al. 2018). As the pathogen continues to spread and the disease intensifies, the populations currently less affected may too follow the same trajectory as those to the north if effective management intervention is not pursued.

***Figure 1:** The high elevation five-needle pines. Clockwise from the upper left: Great Basin bristlecone pine (*Pinus longaeva*), Rocky Mountain bristlecone pine (*P. aristata*), foxtail pine (*P. balfouriana*, photo: Wikipedia), whitebark pine (*P. albicaulis*), southwestern white pine (*P. strobiformis*), and limber pine (*P. flexilis*, photo: CT Holtz). Photos by author unless otherwise noted.*

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***Figure 2:*** Cronartium ribicola *fruiting on a limber pine branch and the bole of a Rocky Mountain bristlecone pine tree.*

How do we prioritize limited management resources across these remote and harsh landscapes for the greatest benefit to the five-needle pine species and likelihood of sustaining or restoring ecosystem services into the future? Traditionally, management is focused in the crisis areas, those that have the highest mortality or impact. However, if only crisis areas receive attention, the currently healthy five-needle pine ecosystems will progressively degrade as the pathogen continues to spread. Also, the crisis-centric management approach neglects that management options are often not equally effective under different forest conditions, and successful restoration is less likely in severely impacted areas. Thus, to manage the high elevation white pine species for future persistence and health, one needs to look beyond the crisis areas and across the full spectrum of ecological conditions. Therefore, both Proactive and Restoration Strategies have been developed for the high elevation five-needle pines (Figure 3). Both have the same long-term management goal of sustaining, restoring, and promoting self-sustaining pine populations in the presence of WPBR and other stresses to support ecosystems processes and services into the future (e.g. Schoettle & Sniezko 2007, Keane & Schoettle 2011, Keane et al. 2012, Schoettle et al. 2019b). The opportunities and timing of interventions to achieve this goal are however different. The Proactive Strategy approach focuses on augmenting natural processes in healthy pine populations to increase forest resilience to mitigate WPBR impacts upon invasion such that ecosystem function and services are sustained throughout the naturalization process of the rust. The approach of the Restoration Strategy is to restore the already impacted populations and impaired ecosystems to re-establish natural processes and ecosystems services.

Fortunately, the five-needle pines have some genetic resistance to *C. ribicola* even though they lack a shared evolutionary history with the pathogen. Although the frequency of resistance is very low, it provides the genetic variation on the landscape on which natural selection can operate. Both complete resistance (R gene, qualitative resistance) and partial resistance (slow rusting, quantitative resistance) types exist in the North American five-needle pines, though not all mechanisms appear to be present in all species. Characterizing and screening for resistance to WPBR in the high elevation five-needle pines began with inclusion of these species in cross species comparison in the mid-1900s and have been a focus since the mid-1990s (see summary by King et al. 2010). The screening process includes growing individual tree seedling families for 6-18 months, exposing them to basidiospores of *C. ribicola* under controlled conditions suitable for spore germination and tree infection, and assessing disease development and resistance phenotypes for 4-6 years post-inoculation. Seedlings with resistance can be planted in gardens or used for scion to generate grafted material for clone banks. In addition, the performance of the progeny can be used to infer the resistance of the seed tree, and that seed tree can be used as scion for grafted material and as a seed source for restoration plantings. If the seed trees are in stands that are not heavily impacted, the baseline genetic frequency of the resistance to WPBR in the sampled population can also be inferred and used in management decisions (Kinloch 1992, Schoettle et al. 2014, 2019a). Identification of resistance in individuals and families may be possible with molecular tools, offering the potential for marker assisted selection (MAS) (e.g. Liu et al. 2016, 2019). The investigations into disease resistance have provided evidence that all the five-needles pines are susceptible to WPBR as well as (1) demonstrating that each species has at least some heritable genetic resistance, (2) seed trees with resistance can be identified to provide seed for restoration plantings or incorporation into breeding programs, and (3) baseline frequency of some genetic resistance traits at the population scale can be estimated for use in management decisions.

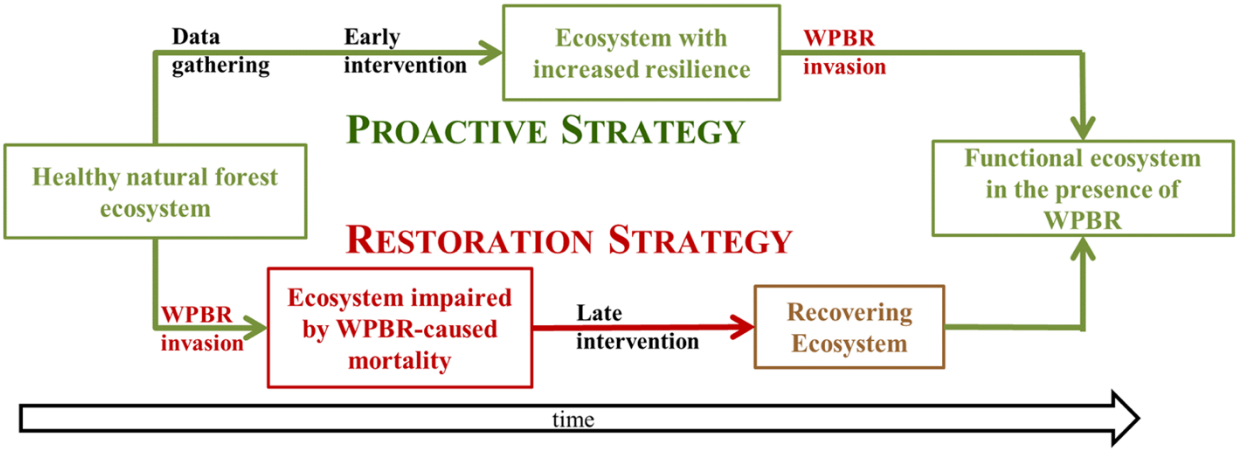

***Figure 3:*** *A schematic of pathways for facilitating sustainable natural forest ecosystems in the presence of a nonnative invasive species.*

Deployment strategies to increase the frequency of genetic resistance in natural forests (as opposed to plantation forests) using regeneration management were not well developed, hence the regeneration for resilience (R4R) framework was advanced to provide a decision structure to prioritize limited resources and utilize natural and artificial regeneration management to offer the best likelihood of success in positioning stands and landscapes to support multi-generational self-sustaining pine populations in the presence of WPBR (Schoettle et al. 2019a). The complexity of natural ecosystems makes mitigating disease impacts more difficult than in managed forests. Sustaining natural forest resilience in the presence of an invasive species requires maintenance of the host population's recovery and adaptive capacity on a landscape spatial and multi-generational temporal scale. Regeneration management, whether it be planting genetically resistant seedling stock, maintaining and augmenting the size of host species populations, or generating a diverse mosaic of stand ages across a landscape, can foster forest adaptation and mitigate impacts caused by invasive species such as *C. ribicola*. Natural regeneration offsets mortality and is therefore a stabilizing force in forests. Regeneration also provides the individuals and genetic combinations to support the dynamic natural selection for genetic resistance and other traits important for long-term adaptation in a changing climate.

Positioning forests for accelerated adaptation to new conditions while sustaining viable population sizes will promote resilience (Schoettle & Sniezko 2007, Keane & Schoettle 2011). Therefore, the management approach must incorporate a long-term and evolutionary perspective which also incorporates adaptation to climate change. Unfortunately for the high elevation five-needle pines, generation time is very long, and the stressors directly affect all nodes of the regeneration cycle (Figure 4). The high elevation five-needle pines are not well equipped for rapid adaptation to novel stresses such as those imposed by the introduction of *C. ribicola* in a changing climate. Reducing the effect of disease on survival and fecundity by increasing heritable disease resistance is essential to sustaining many of these populations and ecosystems services. Numerous trials are underway to discern geographic patterns of genetic WPBR resistance to identify parent trees for seed collections and populations for protection. Ultimately, an increase in the frequencies of resistance in populations over the landscape will help establish a new equilibrium from which pine species and associated ecosystems will have the best opportunity to exist and function in the presence of the permanent residence of *C. ribicola*.

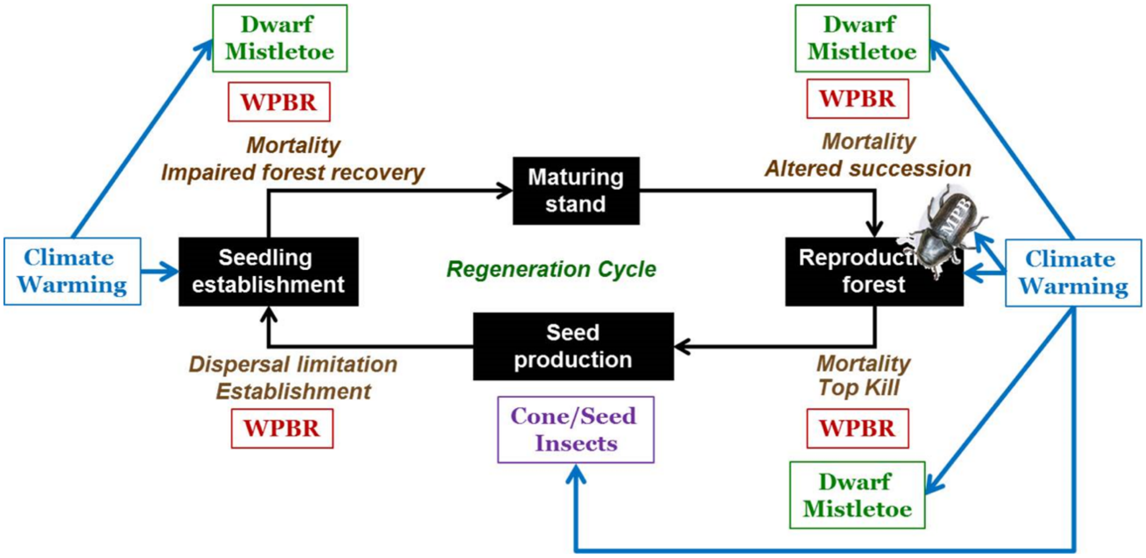

***Figure 4:*** *High elevation five-needle pine regeneration cycle and damage agents (from Schoettle et al. 2019b). White pine blister rust (WPBR) affects the regeneration cycle at all points (brown text), while mountain pine beetle (MPB) only impacts larger mature trees, and cone and seed insects only impact seed production. Dwarf mistletoe can infect trees of all ages. Climate change may directly impact insect populations, seedling establishment, and tree growth and may indirectly affect other interactions within the cycle.*

Ecological condition and context determine the likelihood of success of management interventions to mitigate impacts of WPBR. In populations heavily impacted by WPBR, the remaining seed trees may be too few to support natural regeneration even with management intervention. Likewise, rust pressure can be so high that it will overcome the expression of WPBR-resistance, reducing the efficacy of artificial regeneration with resistant seedling stock as well. Management has a low probability of successfully rebuilding a population under these conditions; triaging these areas in favor of managing areas with less rust pressure may be a better investment. In threatened but currently healthy populations, interventions to increase natural regeneration can help increase population size while early planting of seedling stock with disease resistance traits will enable them to begin to mature and offset mortality of the reproductive overstory trees as the disease intensifies over time. The R4R framework encourages looking beyond the crisis areas for other opportunities to conserve the species and prevent population extirpation.

These interventions entail integrating genetic resistance information into an ecological context to prioritize and inform regeneration interventions to restore and sustain healthy landscapes. The R4R decision framework highlights opportunities across stand conditions and discourages management in areas where management has a low probability of success (Schoettle et al. 2019a). The framework has been applied to prioritize management within target regions and can also be applied across a full species range to prioritize actions by probability of success to prevent species extinction. The framework was used in the development of the Proactive Limber Pine Conservation Strategy in the Greater Rocky Mountain National Park Area (Schoettle et al. 2015, 2019b) and prioritizing treatments for a Restoration Strategy for whitebark pine in a pilot area within the Crown of the Continent Ecosystem (Jenkins et al. 2019).

Rocky Mountain National Park (RMNP) is at the infection front for *C. ribicola* in Northern Colorado, and the park has a responsibility to prevent ecosystem impairment. The Proactive Limber Pine Conservation Strategy for the Greater Rocky Mountain National Park Area is an outcome of a partnership between RMNP and the USDA Forest Service. The Strategy focuses on timing specific monitoring and interventions efforts to inform management to sustain healthy limber pine populations and ecosystems during invasion and naturalization of WPBR, thereby putting limber pine on a trajectory that does not lead to ecosystem impairment in the future (Schoettle et al. 2015, 2019b, Cleaver et al. 2017). During this collaboration, a high frequency of complete resistance to WPBR in limber pine populations in RMNP and surrounding areas was discovered revealing a unique feature of this area’s ecology (Schoettle et al. 2014). That we have this information and the other site-based genetic and disturbance ecology information before the limber pine populations have been invaded by WPBR is unique. This situation justified developing a conservation strategy specific to the greater RMNP area.

The major focal areas for management activities are (1) Promote ex situ and in situ conservation - continue and expand efforts to collect and archive limber pine genetic diversity through seed collections and protect limber pine trees from mountain pine beetle, WPBR, and fire to minimize mortality when and where land designations and management objectives permit; (2) Increase population size and sustain genetic diversity - increase the number of limber pine trees on the landscape through planting or seeding, or both, immediately to offset future mortality and to sustain viable self-sustaining populations; (3) Locate treatments to maintain durability of complete WPBR resistance - minimize selective pressure on the rust by planting trees with a range of susceptibilities only in low-WPBR-risk areas to reduce the probability of the proliferation of genotypes virulent to the complete resistance in limber pine; (4) Discover, develop, and deploy local quantitative WPBR-resistant sources - research quantitative (polygenic) WPBR resistance types in limber pine in the greater RMNP area and establish a clone bank of these genotypes (which can be protected from fire and other stresses) to provide seed for future plantings, and (5) Monitor pines and rust - Monitor for limber pine health, early detection of WPBR, and WPBR virulence. The Proactive Conservation Strategy was adopted by the park in 2015 and has served as a model for ongoing proactive conservation efforts for Rocky Mountain bristlecone pine, Great Basin bristlecone pine, as well as southwestern white pine and can be applied to other healthy portions of the distribution of limber pine and for those for foxtail pine and whitebark pine also.

The R4R framework has also been applied to the very heavily impacted area of the Crown of the Continent Ecosystem (CCE - northern Montana, southern British Columbia and Alberta; https://www.crownmanagers.org/five-needle-pine-working-group). The decision space is constrained by high impacts, but the landscape can still be stratified by forest condition and context to allocate treatment resources to have the highest likelihood of restoring whitebark pine within the CCE. It is well documented that five-needle pine forests in the CCE have declined significantly due to a combination of biotic and abiotic stressors. The High Five Working Group of the Crown Managers Partnership was formed to prioritize and advance collective efforts to effectively monitor, conserve, and restore five-needle pines across the complex jurisdictional boundaries of the CCE. The working group developed a proof of concept methodology for whitebark pine restoration within a US portion of the CCE (Jenkins et al. 2019). Future applications will cover the full CCE including both whitebark and limber pine. The strategy integrates existing data sources, expert opinion, and modeling within a GIS platform. Areas were first identified where whitebark pine is capable of existing on the landscape and then rated related to (1) conservation value as defined as those areas currently providing ecosystem services or capable of providing ecosystem services and then (2) the threats/stressors that have reduced, or are likely to reduce, conservation value were identified and quantified. Restoration actions were then prioritized based on the likelihood of successfully benefitting whitebark pine under each of the stressor's levels such that areas where stressors negatively impacted the potential success of the treatments were assigned a lower priority using an adaptation of the R4R framework. Finally, all factors were integrated, and priority core areas are defined for focused restoration in the future. The outcome of this effort is a draft methodology to identify priority areas for restoration within the CCE and for the National Whitebark Pine Restoration Strategy within the USA and can serve as a model for restoration strategies for other areas impacted by WPBR including the northern distributions of whitebark, limber, and foxtail pines.

Timely management approaches that incorporate both ecological context and an evolutionary perspective increase the likelihood of successfully sustaining high-mountain pine ecosystems into the future. The R4R framework highlights opportunity for increasing the resilience of both WPBR-threatened and impacted high elevation five-needle pines ecosystems across western North America. In healthy but threatened ecosystems, acting now will increase forest resilience to position the ecosystems to develop fewer impacts, and need less restoration, in the future, and the R4R framework encourages managers to not wait until the ecosystems are impaired to begin managing for increased resilience. In impacted systems, R4R recommended prioritizing management in locations that have the greatest likelihood of successfully restoring function and to look for opportunities beyond the heavily impacted areas that often attract most of the attention but have a poor prognosis. The same framework can be used when trying to prevent range-wide species endangerment by prioritizing treatments across a diversity of current stand conditions and WPBR hazards to increase the likelihood that at least some populations avoid extirpation. When prioritizing limited resources, context is critical. Accepting that some habitats may not support sustainable populations in the future, even with intervention, is essential for making good treatment investment decisions. The future of the high elevation five-needle pine ecosystems may depend on it.

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# ECOLOGY AND EVOLUTION OF A TREE SPECIES CHALLENGED BY DUAL THREATS

**Kristen Waring[1], Sam Cushman[2], Andrew Eckert[3], Lluvia Flores-Renteria[4], Richard Sniezko[5],
Christopher Still[6], Christian Wehenkel[7], Amy Whipple[1], Michael Wing[6], Justin Bagley[3], Ethan Bucholz[1],
Socorro Gonzalez-Elizondo [8], Marja Haagsma[6], Jessica Hartsell[1], Cory Garms[6], Evan Heck[5],
Jeremy Johnson[1,5], Erin Landguth[9], Alejandro Leal Sáenz[7], Mitra Menon[3], Ehren Moler[1], Gerald Page[6],
John Shaw[2], Andrew Shirk[10], and Jared Swenson[1]**

## Abstract

We are studying the ecological and evolutionary processes affecting the geographical distribution of southwestern white pine (SWWP; *Pinus strobiformis*), an important tree species of the Southwestern USA and Mexico. Southwestern white pine viability is threatened by changing climate and an invasive tree disease, white pine blister rust (caused by the fungal pathogen, *Cronartium ribicola*). Rapid climate change is negatively affecting SWWP by increasing heat and drought stress, and thus challenging its ability to adapt. White pine blister rust causes extensive tree decline and mortality, including in SWWP. The dual threats of climate change and invasive species make forecasting future tree distributions at continental scales an urgent challenge. The goal of our project is to determine how gene movement among populations, adaptation to disease and drought, heritable changes beyond DNA mutations, and a changing environment interact to govern the success of SWWP. We are utilizing and developing tools to help forecast and manage the future of the species, including those from genomics, common gardens, tree disease resistance testing, engineering, and technology innovation to measure drought tolerance and physiological response. Results will be synthesized and included in cutting-edge landscape genomics models to meet our overarching goal. We will present our framework and current project status, including results. Completed products include development of landscape genomic models, a species distribution model incorporating climate change, and adaptive trait, phenotype plasticity, and genomic variation analyses. Continuing work incorporates these aspects in addition to transgenerational plasticity research and a genome-wide association study.

**Broad Project Goals:**

1. Find genetic resistance to white pine blister rust
2. Find adaptive traits using common gardens
3. Find adaptive genes using next generation sequencing
4. Link phenotypes to genotypes using remote sensing technology
5. Model gene flow, adaptive variation, and climate change across the landscape

**Acknowledgements:** This material is based upon work supported by the National Science Foundation (NSF) under Grants No. EF-1442597, EF-1442486 and EF-1442456. The Southwest Experimental Garden Array (SEGA) project is supported by NSF Grant No. 1126840 and the Merriam Powell Center for Environmental Research. Dorena Genetic Resource Center also receives support from the USDA Forest Service Region 6 Genetic Resource Program and Forest Health Protection. Additional financial support was provided by the NAU Technology Research Initiative Fund and the USDA Forest Service Gene Conservation Program. We thank the many seasonal staff, volunteers, and collaborators who have made this work possible through their support.

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**Cone, seed, and foliage source locations (Figure 1).**

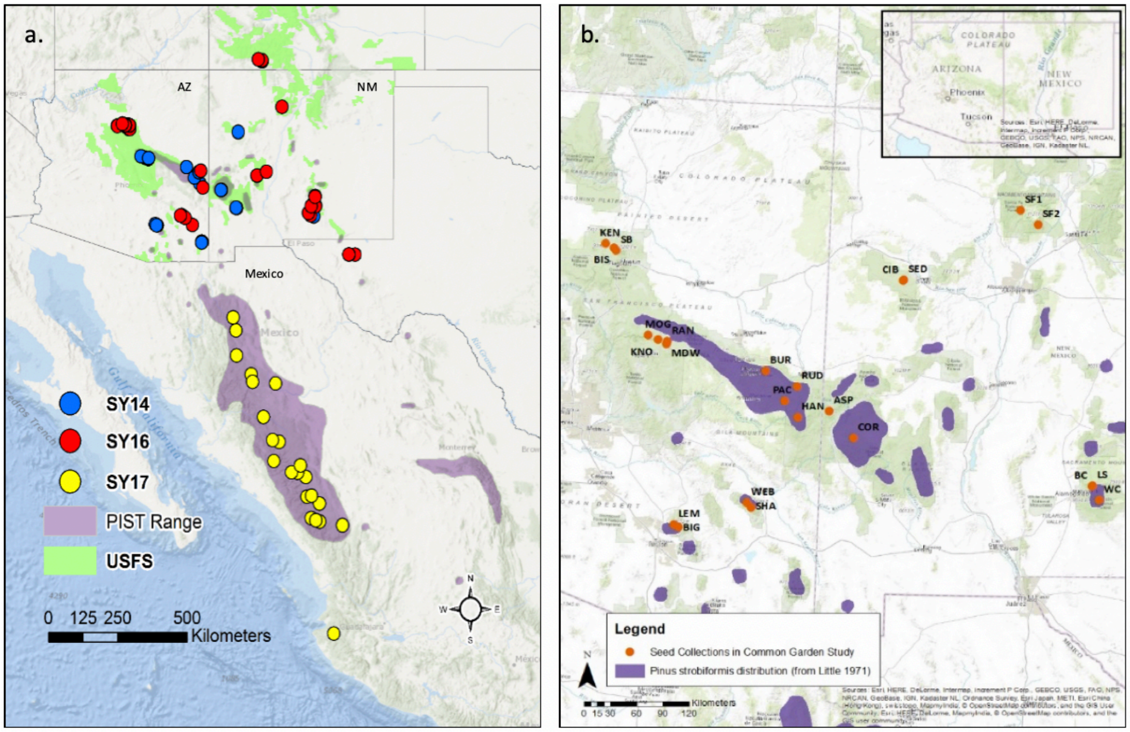

***Figure 1: a.** Sow year 2014, 2016 and 2017 source population locations. **b.** Sow year 2015 source population locations.*

### Goal 1: Find genetic resistance to white pine blister rust

Resistance screening is taking place for both major gene resistance (MGR) and quantitative resistance. Results are preliminary, with ongoing inspections and results being updated multiple times annually. We have identified new maternal trees with MGR in Arizona and New Mexico plus a potential source tree in Mexico. Early results indicate that genetic resistance is greater in more southern US populations, but these are preliminary (Figure 2). Additionally, clustering of genetic resistance in south-central New Mexico corresponds to the general area white pine blister rust was first identified in southwestern white pine, providing the most time for natural selection against susceptible families to occur.

### Goal 2: Find adaptive traits using common gardens

We are using three common gardens within the (SEGA) (Figure 3) to grow progeny side by side, simulate climate warming using an elevational gradient, and compare adaptive traits across families and populations. Starting in 2018, half of the seedlings are subject to a drought experiment during the growing season. Each SEGA site is equipped with a full weather station, fencing to protect from browse damage, and irrigation system. Seedlings were planted in a complete randomized design, in raised bed boxes in 10 rows of 10 during late summer, in 2015, 2016, and 2017. Seedlings are measured bi-annually to assess growth (height and diameter), phenology, and mortality. A subsample has been included in seedling physiology research as well.

*Unpublished results:* Mortality has been highest in the high elevation garden but similar between the mid and low elevation gardens. Results from seedling physiological studies indicate some plasticity in response to increased temperature (carbon isotopes and stomatal densities) at the low elevation garden and a drought avoidance water use strategy.

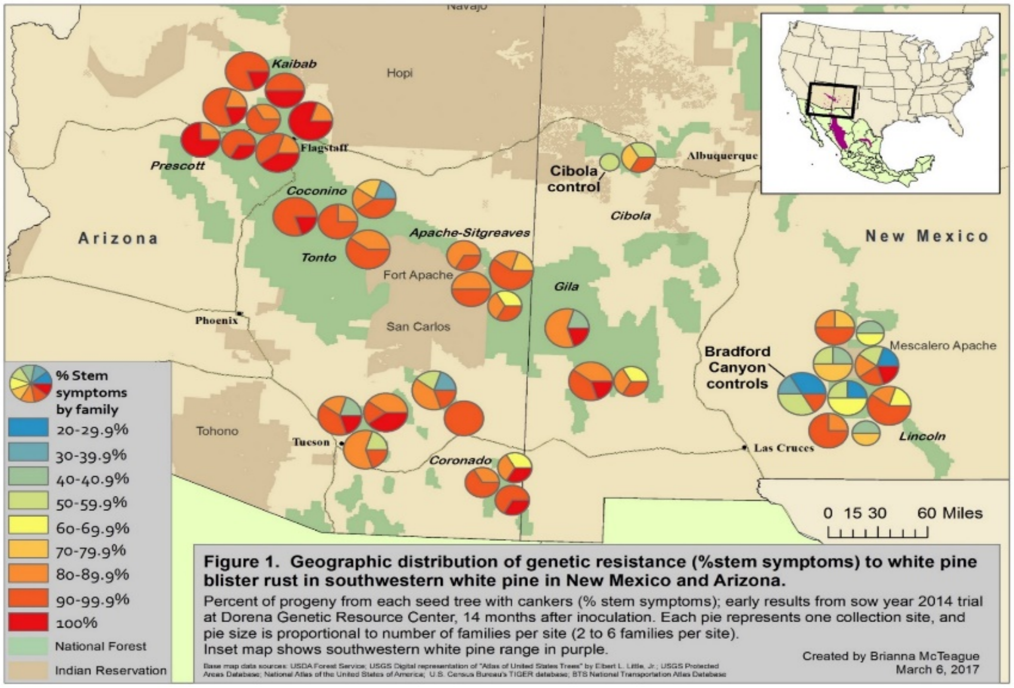

**Figure 1. Geographic distribution of genetic resistance (%stem symptoms) to white pine blister rust in southwestern white pine in New Mexico and Arizona.**

Percent of progeny from each seed tree with cankers (% stem symptoms); early results from sow year 2014 trial at Dorena Genetic Resource Center, 14 months after inoculation. Each pie represents one collection site, and pie size is proportional to number of families per site (2 to 6 families per site).
Inset map shows southwestern white pine range in purple.

Created by Brianna McTeague
March 6, 2017

Base map data sources: USDA Forest Service; USGS Digital representation of "Atlas of United States Trees" by Elbert L. Little, Jr.; USGS Protected Areas Database; National Atlas of the United States of America; U.S. Census Bureau's TIGER database; BTS National Transportation Atlas Database

***Figure 2:*** *Preliminary results (March 2017) indicating geographic locations of genetic resistance in the US Southwest. Unpublished data, Dorena Genetic Resource Center, Cottage Grove, Oregon.*

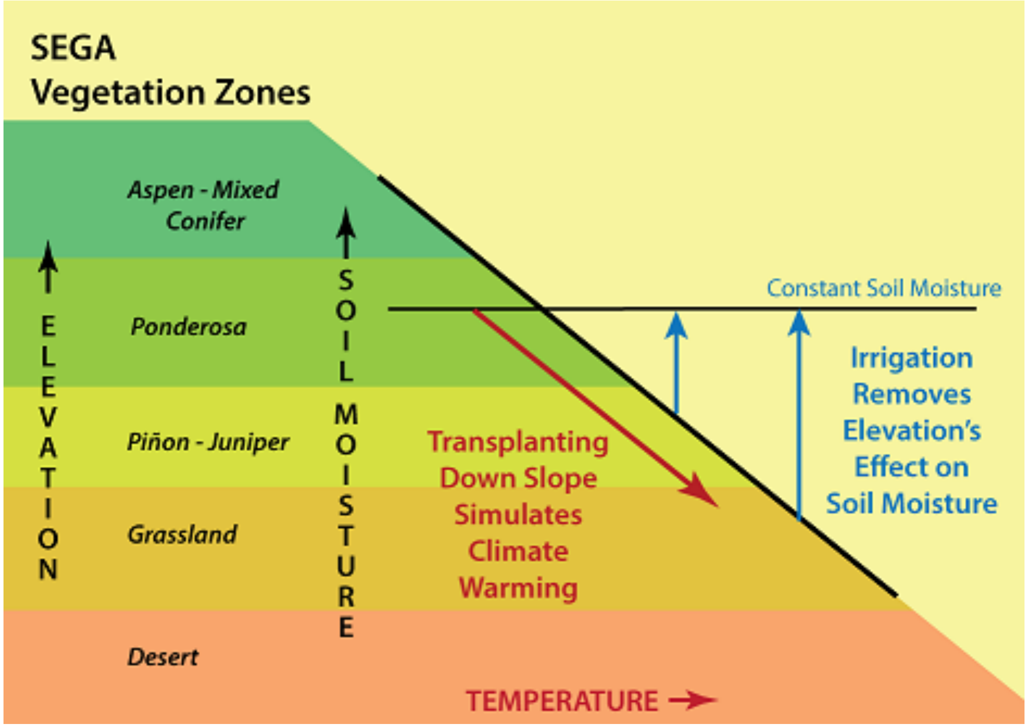

***Figure 3:*** *Environmental gradients and experimental design of the Southwest Experimental Garden Array (SEGA). This study is utilizing the aspen-mixed conifer (high elevation), ponderosa (mid elevation), and piñon-juniper (low elevation) common garden sites.*

### Goal 3: Find adaptive genes using next generation sequencing

Research related to this goal has thus far been focused on investigating the genetic relationship between *Pinus flexilis* (limber pine) and *P. strobiformis* (SWWP). The US populations of SWWP primarily fall into a hybrid zone, with trees containing genes from both species (weighted toward SWWP) (Figure 4, from Menon et al. 2018). Additional research found evidence that the hybrid zone is shifting north (Menon et al. 2019).

### Goal 4: Link phenotypes to genotypes using remote sensing technology

The team is using a multispectral sensor to image white pine blister rust inoculated and un-inoculated seedlings to determine if disease infection can be detected remotely. Preliminary results were positive and led to a successful Special Technology Development Program proposal to expand this component of the research.

### Goal 5: Model gene flow, adaptive variation, and climate change across the landscape

To date, the landscape genomics models have been updated and validated, and in the coming year, data from the other four goals will be used to initiate the model. Additionally, we predicted the distribution of southwestern white pine under the changing climate (Figure 5); the species distribution model will also be a component of this modeling effort.

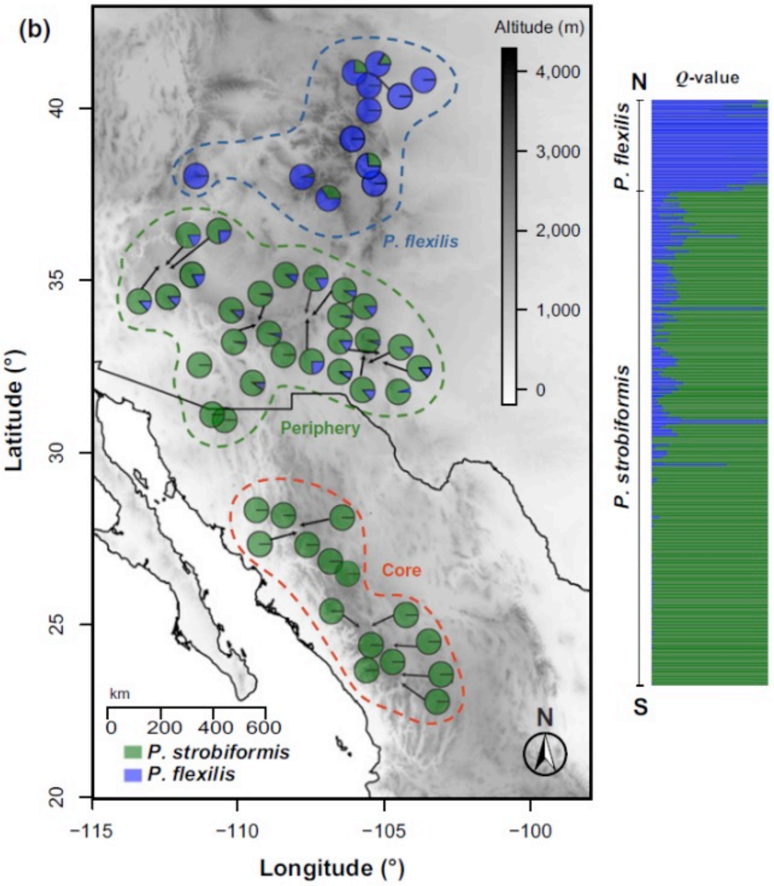

***Figure 4:*** *Geographic locations of core southwestern white pine populations (red dashed line), peripheral populations in the hybrid zone (green dashed line), and limber pine populations (blue dashed line). Pie charts represent ancestral proportions of* P. strobiformis *(green) and* P. flexilis *(blue); also represented by the Q-value bar chart on the right. From Menon et al. 2018.*

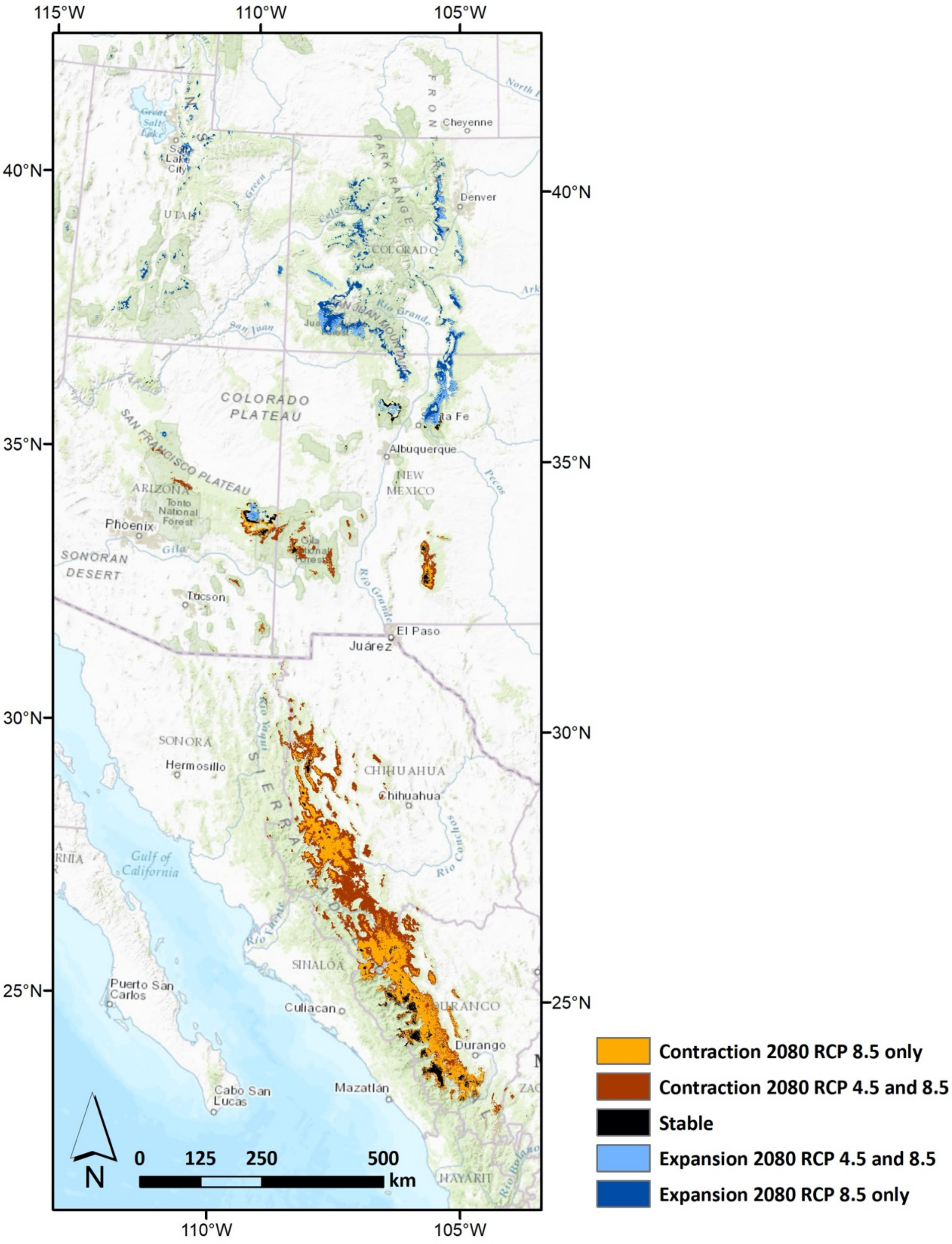

***Figure 5:*** *Predicted areas of southwestern white pine range expansion, stability, and contraction in 2080 under two climate scenarios. From Shirk et al. 2018.*

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# PANEL: TOOLS FOR LANDSCAPE EVALUATIONS (MODERATOR: BRENT OBLINGER)

# UTILIZING MULTIPLE LANDSCAPE-LEVEL DETECTION AND MODELING TOOLS FOR MANAGEMENT OF SUDDEN OAK DEATH IN OREGON

**Sarah Navarro[1], Devon Gaydos[2], Alan Kanaskie[1], Danny Norlander[1], Eric Rounds[3], Mark Riley[4], and Zach Heath[5]**

Sudden Oak Death (SOD), caused by *Phytophthora ramorum*, is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens this species throughout its range in Oregon. In July 2001, the disease was first discovered in coastal southwest Oregon forests. Since 2001, an interagency team has attempted to eradicate and slow the spread of disease through a program of early detection, survey and monitoring, and destruction of infected and nearby host plants. Survey, detection, and monitoring efforts compose of ground, aerial, and stream bait surveys. Ground-based detection and delimitation surveys around infested sites are conducted year-round. Aerial surveys, using both fixed-winged and helicopter aircraft, are conducted four times per year; the main surveys occur in July and October when current-year mortality is most visible. Aerial surveys cover a cumulative area of at least 700,000 acres of forest; ground surveys cover 600 acres. In order to streamline the processing of field samples, the program has begun utilizing ESRI ArcGIS programs for electronic data collection and information sharing between stakeholders. The current program is incorporating the use of high-resolution, digital aerial imagery as a means to augment aerial surveys. A comparison of past aerial survey results with the high-resolution imagery demonstrates the overall accuracy of the aerial surveys. Oregon's SOD Program is currently working with US Forest Service remote sensing specialists to create an automated workflow to identify declining and dead tanoak trees from the aerial imagery to increase the efficacy of early disease detection. Lastly, in 2017, a SOD Task Force convened local, state and federal government agencies, tribes, industry, local residents, and environmental groups. The mission of the Task Force was to develop a collaborative-based strategic action plan, including securement of additional resources to contain the NA1 strain of *Phytophthora ramorum* and eradicate the EU1 strain of *Phytophthora ramorum* in Curry County, Oregon using the best available science. Recently, task force members took part in a participatory modeling workshop to evaluate landscape-scale management strategies for the EU1 strain (Gaydos et al. 2019). Following the success of the workshop, the SOD Task Force is interested in using the tool for future policy and funding discussions.

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ROCKY MOUNTAIN
NATIONAL PARK

# THE 20-YEAR FOREST HEALTH STRATEGIC PLAN FOR EASTERN WASHINGTON

**Chuck Hersey[1], Derek Churchill[1], and Amy Ramsey[1]**

Forest health is defined in Washington state statute as “the condition of a forest being sound in ecological function, sustainable, resilient, and resistant to insects, diseases, fire and other disturbances, and having the capacity to meet landowner objectives” (RCW 76.06).

According to this definition, broad swaths of eastern Washington forestland are in an unhealthy state. An analysis by The Nature Conservancy and the U.S. Forest Service identified 2.7 million acres of forestland in Central and Eastern Washington requiring natural disturbance or active management to create forest structures more resilient against insects, diseases, and wildfires (Haugo et al. 2015).

In 2016, the Legislature directed the WA Department of Natural Resources (DNR) to develop a forest health strategic plan to “treat areas of the state forestland that have been identified by the department as being in poor health.” DNR determined that to meet the intent of the Legislature, and to address the forest health issue in a meaningful way, it was necessary to take a broad view of “treat areas of state forest lands,” and to adopt a guiding philosophy of “all lands, all hands.” This DNR guiding philosophy means the agency aims to address forest health issues at a landscape-scale and in coordination with all landowners to ensure forest health treatments advance in a coordinated, strategic fashion.

The 20-Year Forest Health Strategic Plan is the high-level framework guiding the State of Washington’s work and investments to improve forest health, help forests adapt to projected climatic changes, and achieve forest-related ecological, economic, and social benefits in Central and Eastern Washington. The overarching strategy is to maximize the effectiveness of forest health treatments by coordinating, planning, prioritizing, and implementing forest management activities across large landscapes.

Washington’s 20-Year Forest Health Strategic Plan sets a goal of treating 1.25 million acres over the next 20 years to improve the resilience of forests in eastern Washington. The authority and direction contained in Senate Bill (SB) 5546 guides DNR’s efforts to improve forest health across all ownerships in large landscapes. SB 5546 requires DNR to create a Forest Health Assessment and Treatment Framework that assess a minimum of 200,000 acres of fire prone lands each biennium and identifies forest health treatment needs across all lands. SB 5546 also provides legislative direction and tools to help achieve the state’s treatment goals across all lands.

The first step of the Forest Health Assessment and Treatment Framework was to select which priority watersheds the state will analyze for forest health treatment needs across all lands and focus its forest health investments. DNR identified its priority planning areas based on a data-driven analysis of HUC 6 (Hydrologic Unit Code) watersheds in the region, as well as feedback from forest collaboratives, tribes, relevant federal and state agencies, the Forest Health Advisory Committee, and other stakeholders (Figure 1). DNR selected 12 forest health planning areas for the 2018 planning cycle to analyze for forest health treatment need (Figures 2 and 3). An additional 21 forest health planning areas were selected for the 2020 planning cycle and will be analyzed in 2019 and 2020 (the 2020 planning cycle) with results reported by December 2020. The 2018 forest health planning areas contain over 1 million acres of forestland and the 2020 planning areas contain over 1.65 million acres of forestland.

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[1] *Washington Department of Natural Resources, Forest Health and Resiliency Division, Olympia, WA*

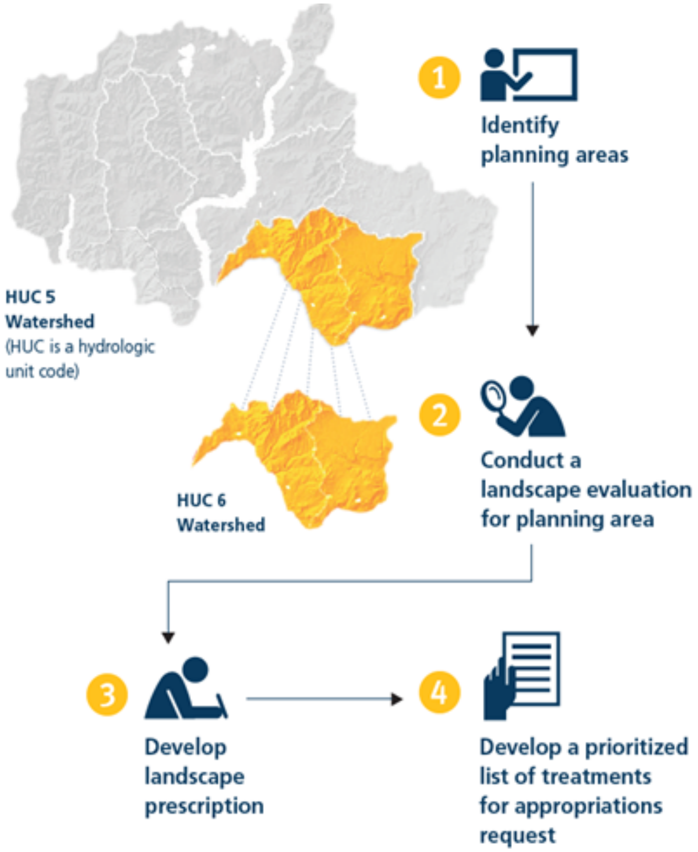
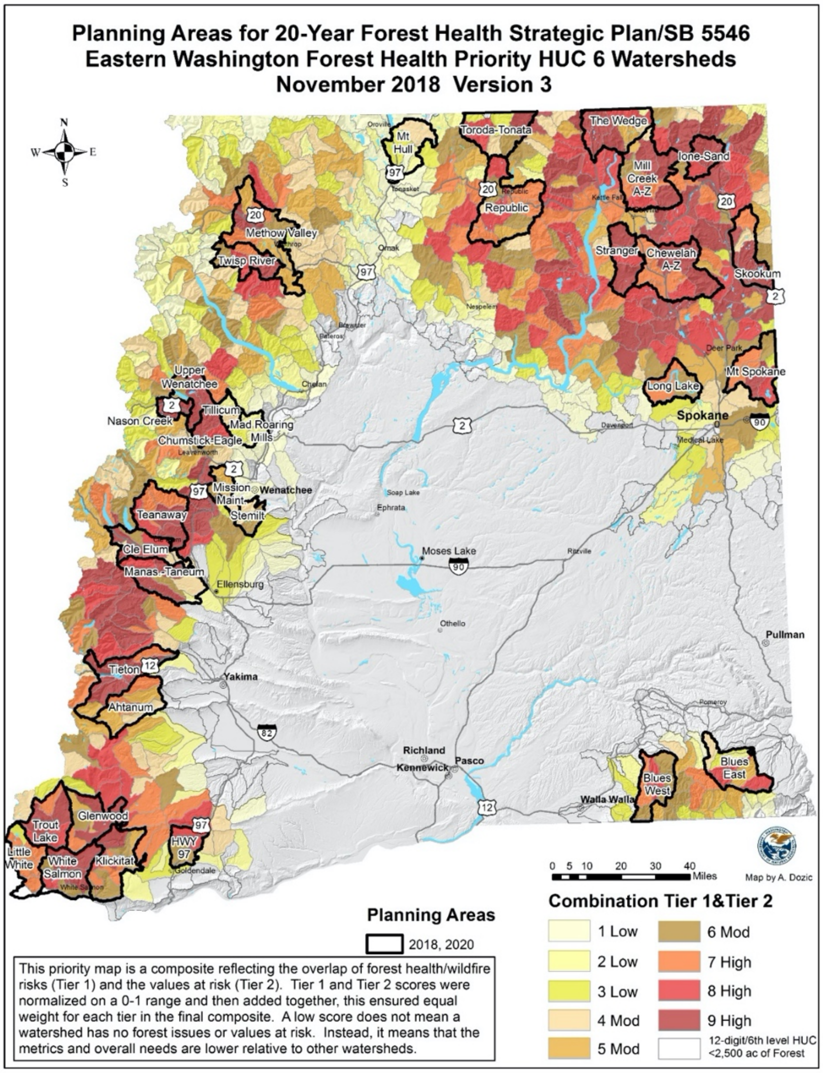


***Figure 1:*** *Major Steps of SB 5546 Forest Health Assessment and Treatment Framework to Accomplish the Treatment Goals of the 20-Year Forest Health Strategic Plan.*

For the 2018 planning areas, DNR conducted landscape evaluations to assess forest health conditions and determine treatment needs across all lands. A landscape evaluation is a data driven approach to understanding the current condition of a landscape and its level of resilience to future natural disturbances, including climatic change. A primary result of the landscape evaluation is a summary of vegetation changes relative to historical reference conditions, current fire and drought risk, and wildlife habitat needs. The information and data from a landscape evaluation is then synthesized into a landscape prescription that describes and quantifies the shifts in vegetation conditions and pattern that are needed to move the landscape into an ecologically resilient condition and significantly reduce fire risk to communities.

DNR is employing the landscape evaluation and prescription process to assess the forest health treatment needs in the forest health planning areas as required by SB 5546. The purpose of the landscape evaluation and prescription is to set high-level forest health treatment targets for each planning area so that the state, landowners, and stakeholders understand the level of treatment needed to create resilient forest conditions, work together to implement landscape-scale treatments, and provide a benchmark to track progress on achieving treatment goals. Landscape evaluations and prescriptions do not mandate treatment targets or types for specific landowners. Instead, they provide recommendations and benchmarks for the planning area as a whole. Individual landowners then conduct their own field assessment, planning, and decision-making processes to determine the treatments they can implement to achieve overall landscape goals while meeting their own management objectives and regulatory requirements.


Planning Areas for 20-Year Forest Health Strategic Plan/SB 5546
Eastern Washington Forest Health Priority HUC 6 Watersheds
November 2018 Version 3

This priority map is a composite reflecting the overlap of forest health/wildfire risks (Tier 1) and the values at risk (Tier 2). Tier 1 and Tier 2 scores were normalized on a 0-1 range and then added together, this ensured equal weight for each tier in the final composite. A low score does not mean a watershed has no forest issues or values at risk. Instead, it means that the metrics and overall needs are lower relative to other watersheds.

Map by A. Dozic

***Figure 2:*** *2018 and 2020 Planning Areas for the 20-Year Forest Health Strategic Plan and SB 5546, and Forest Health HUC 6 Watersheds Priority Rankings.*

Based on the landscape evaluations and prescriptions for the twelve 2018 planning areas, DNR estimates that 286,220 - 430,120 acres of treatments are needed to move these landscapes into a resilient condition (Table 1). Across all of the 2018 planning areas, this equates to treating approximately 30-40% of the forested area.

A combination of mechanical treatments, prescribed fire, and managed wildfire will be needed to accomplish the identified treatment needs. Based on tree size class and canopy cover information from the landscape evaluations, the majority of the acres needing forest health treatments are commercially viable, although commercial viability ultimately depends on multiple factors. This means the cost of mechanically treating the forest stand can be covered by the revenue generated from the trees removed from the stand and potentially generate some revenue to help cover some costs of follow-up treatments such as prescribed fire. However, individual landowners will determine treatment types by taking into account their on-the-ground conditions, objectives, and constraints.

***Table 1:*** *Forest Health Treatment Needs for the 2018 Forest Health Planning Areas.*

| **Planning Area** | **Forest Structure Class (acres)** | | |
|---|---|---|---|
| | **Small Dense[1]** | **Medium-Large Dense[2]** | **Large-Medium Open[3]** |
| Chewelah A-Z | 2,000 - 3,500 | 45,500 - 66,500 | 3,500 - 8,000 |
| Mill Creek A-Z | 1,000 - 2,000 | 54,000 - 72,000 | 2,000 - 6,000 |
| Mt Spokane | 500 - 1,000 | 21,000 - 29,000 | 4,000 - 8,500 |
| Upper Wenatchee | - | 15,000 - 25,000 | 500 - 2,000 |
| Stemilt | - | 6,200 - 7,900 | 3,000 - 5,700 |
| Manastash-Taneum | 3,500 - 6,500 | 11,000 - 19,000 | 2,000 - 4,000 |
| Cle Elum | 1,500 - 3,000 | 14,000 - 20,000 | 2,500 - 5,500 |
| Ahtanum | 2,000 - 2,500 | 13,000 - 18,500 | 4,000 - 8,000 |
| Trout Lake | - | 17,500 - 31,000 | 1,000 - 2,000 |
| White Salmon | 500 - 1,000 | 35,000 - 48,000 | 2,500 - 6,000 |
| Total | 11,000 - 19,500 | 232,200 - 336,900 | 25,000 - 55,700 |
| **Subtotal** | **268,200 - 412,100 acres** | | |
| Tillicum | 7,614 acres | | |
| Mission Maintenance | 10,406 acres | | |
| **Grand Total** | **286,220 - 430,120 acres** | | |
| Anticipated Treatment Type | [1] Non-commerical thin + fuels treatment. May also be prescribed fire or managed wildfire in some areas. | | |
| | [2] Commerical thin + fuels treatment where possible. May be non-commercial, prescribed fire, managed wildfire or regeneration harvest in some areas. | | |
| | [3] Maintenance treatments: prescribed fire or mechanical fuels treatments. | | |

The implementation of the forest health treatment needs identified through the landscape evaluation process for each planning area will likely take several biennia to accomplish. The pace and scale of forest health treatment implementation will be driven by some common and unique factors for each planning area such as: ratio of commercial versus non-commercial treatments, forest product markets, access, capacity of land managers and contractors to plan and implement treatments, and funding levels for non-commercial treatments.

The efforts of the 20-Year Forest Health Strategic Plan and the Forest Health Assessment and Treatment Framework are complimentary and additional to the substantial existing forest health work already underway by the U.S. Forest Service, other federal agencies, tribes, state agencies, private landowners, and others. Significant forest health treatments have been completed or planned in the forest health planning areas prior to the creation of the 20-Year Forest Health Strategic Plan and prior to being designated as a forest health planning area. Being designated as a forest health planning area focuses additional resources to help address remaining forest health needs in a collaborative fashion and provides monitoring of forest health conditions to track achievement of landscape-level forest health goals over time.

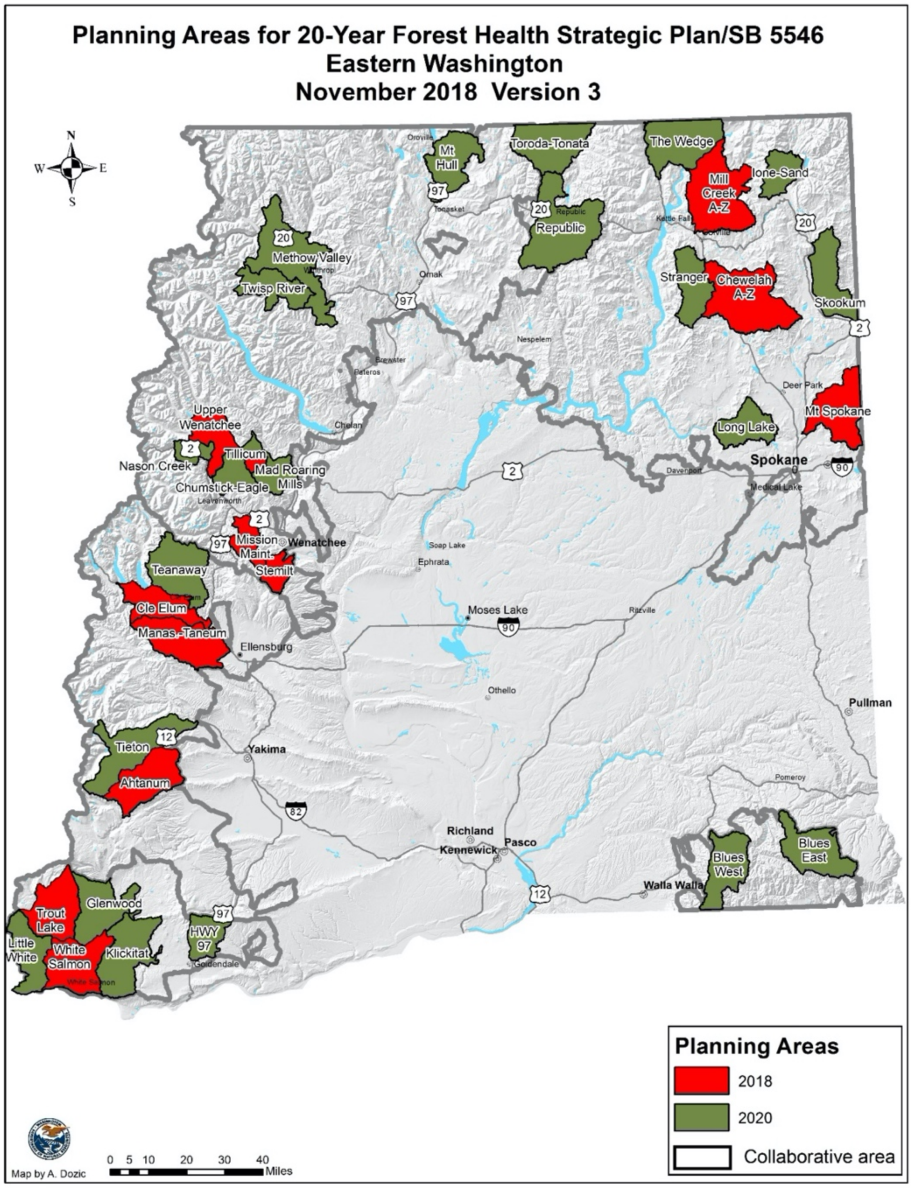


*__Figure 3:__ 2018 and 2020 Planning Areas for the 20-Year Forest Health Strategic Plan and SB 5546.*

## Acknowledgements

We greatly appreciate everyone who has worked on the 20-Year Forest Health Strategic Plan for Eastern Washington.

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# DATA SOURCES AND METHODS TO IMPROVE ROOT DISEASE RISK MODELS FOR BROAD SCALE ASSESSMENTS

**Mike Simpson[1] and Brent Oblinger[1]**

## Background

Root diseases are predicted to cause mortality and losses into the future in the contiguous U.S. that exceed predicted losses from many other groups of forest diseases and insects (Krist et al. 2014, Lockman & Kearns, eds. 2016). Risk models have often incorporated measures of host abundance and susceptibility, environmental variables such as temperature and precipitation, and known occurrences of diseases. An overview was presented of existing West Coast Armillaria and laminated root disease models used in the U.S. Forest Service's 2012 and 2018 National Insect and Disease Risk Maps (NIDRM, Krist et al. 2014), and we suggest new approaches using improved vegetation/host estimates, available remote sensing products that improve the identification of existing root disease centers, and subsequent estimates of the risk of mortality in host species at a finer scale. Proposed new model inputs utilize Gradient Nearest Neighbor (GNN) imputations (Ohmann et al. 2002) of regional Continuous Vegetation Survey and Forest Inventory & Analysis (FIA) vegetation plot data to the entire Landsat imagery time series (1984-2017). The intensified plot grid in the Northwest can improve imputations. Use of multiple imputations of vegetation plot data (one/Landsat image year) normalized using LandTrendr (Figure 1; Kennedy et al. 2010) allows a retrospective look at rates of change back to 1984 in multiple forest attributes. In 2011, Meigs, Kennedy, and Cohen found a relationship between LandTrendr spectral trajectories and annual Aerial Detection Survey data for mountain pine beetle and western spruce budworm activity in the Oregon Cascades (Figure 2). The slower, chronic forest change associated with tree mortality induced by root disease is not documented during aerial surveys, so instead we propose using change in forest attributes through time as depicted by changes in LandTrendr-normalized GNN time-series forest attributes to enhance detection of root diseases. The most promising attributes after evaluating multiple, known sites with root disease were slow, chronic decline (1-2% loss in canopy cover/year) of susceptible host species as well as the slow, steady increase of snags and down woody debris through time associated with existing canopy gaps created by root diseases (Figure 3). The new Potential Natural Vegetation dataset from Oregon and Washington, developed by the U.S. Forest Service's Area Ecology Program and others, is another potential input to model risk in plant associations where each root disease is prevalent.

## Methods

These changes detected in % host species loss, the increase in snags/acre, and the increase in down woody debris/acre from 1984-2012 were converted to risk values using the risk curves illustrated in Figure 3 and then added to the existing 2012/18 NIDRM model risk attributes. Calculated risk values were then masked by potential vegetation types suitable for the appropriate root disease where susceptible hosts and the disease commonly occur. Model output is % risk of a particular root disease at a 30 x 30 m pixel resolution. Maximum mortality was capped at 30% over the next 15 years for laminated and Armillaria root diseases in the 2012 and 2018 NIDRM models. Mortality (loss) in host species basal area and host species % mortality can be calculated from the final risk value. See the laminated root disease model example below.

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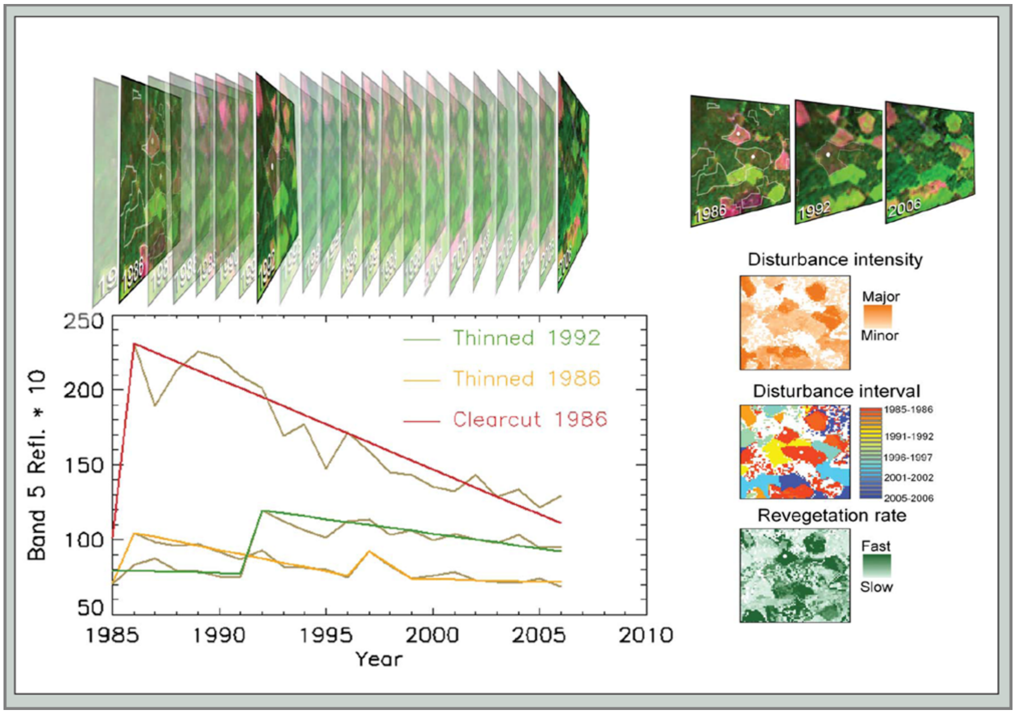
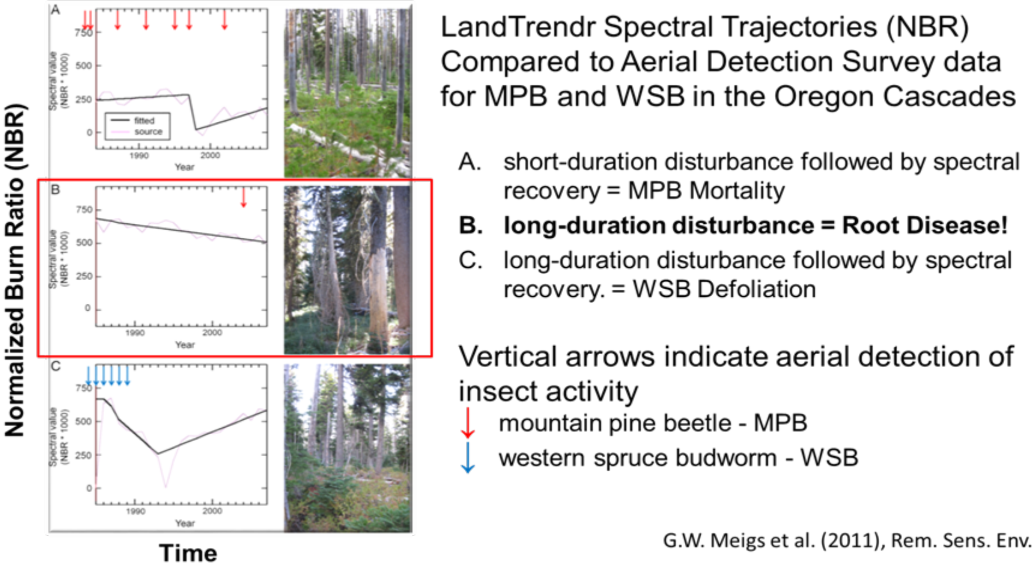


***Figure 1:*** *Landsat Detection of Trends in Disturbance and Recovery (LandTrendr) is a system to standardize Landsat Imagery through time developed by Robert Kennedy and Warren Cohen (Kennedy et al. 2010). It includes temporal normalization and segmentation at the pixel level and minimizes noise from sun angle and vegetation phenology. Segments describe sequences of disturbance and regrowth.*



LandTrendr Spectral Trajectories (NBR) Compared to Aerial Detection Survey data for MPB and WSB in the Oregon Cascades

A. short-duration disturbance followed by spectral recovery = MPB Mortality

**B. long-duration disturbance = Root Disease!**

C. long-duration disturbance followed by spectral recovery. = WSB Defoliation

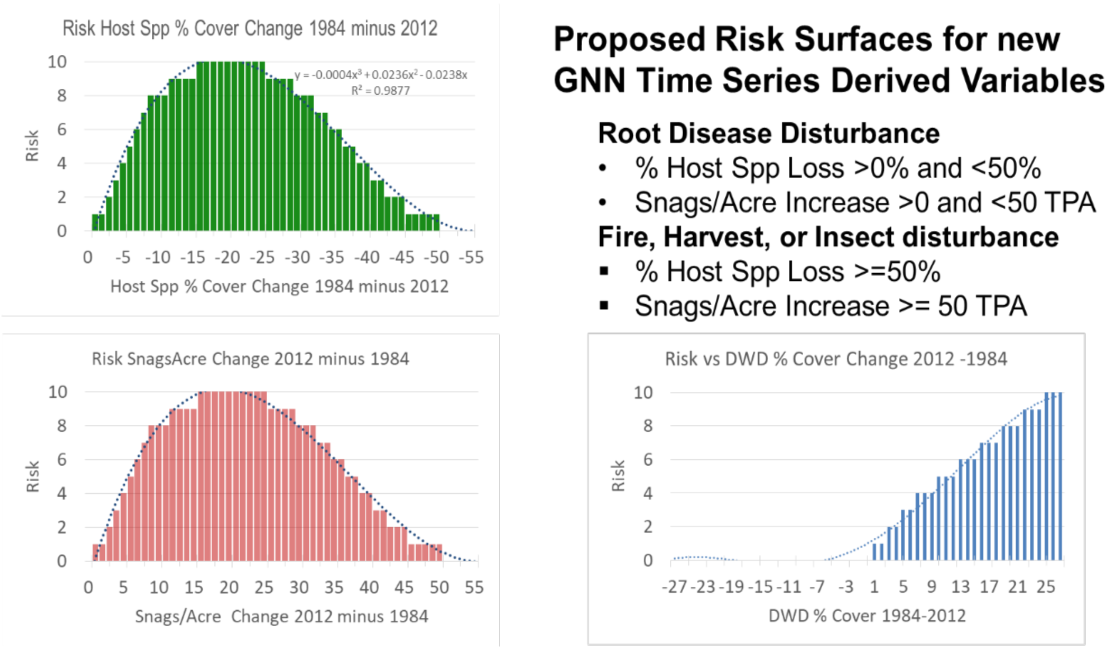
Vertical arrows indicate aerial detection of insect activity

↓ mountain pine beetle - MPB

↓ western spruce budworm - WSB

G.W. Meigs et al. (2011), Rem. Sens. Env.

***Figure 2:*** *Meigs, Kennedy, and Cohen (2011) related LandTrendr spectral segments to Aerial Detection Survey data in the Oregon Cascades and found certain trajectories were associated with damage from mountain pine beetle and western spruce budworm. We propose that patterns in longer-duration, chronic disturbance are associated with mortality induced by root diseases after evaluating patterns from sites where root disease has been confirmed.*



**Proposed Risk Surfaces for new GNN Time Series Derived Variables**

**Root Disease Disturbance**
- % Host Spp Loss >0% and <50%
- Snags/Acre Increase >0 and <50 TPA

**Fire, Harvest, or Insect disturbance**
- % Host Spp Loss >=50%
- Snags/Acre Increase >= 50 TPA

***Figure 3:*** *Proposed risk surfaces for % mortality of host species, and an increase in snags/acre and down woody debris cover through time (1984-2012) based on GNN data. Risk of root disease peaked at about 1% loss in host species cover per year, an increase of 1-2 snags/acre per year, and an increase of 1% in down wood cover per year. Higher rates of change are associated with other disturbances.*

**Laminated Root Disease Modeled Risk:**

Total_LamRisk = HostBasalArea + Host_15inDBH&up + HostCanopyCover + %HostCanopyCovLoss + %ResistantSppCanopyCov + ResistantSpp_5inDBH&lower + SnagsIncrease + DownWoodyDebrisIncrease + MeanAnnualTemp + MeanAnnualPrecip
LamRisk_Final = Total_LamRisk * LamRisk_PotentialNatVegMask
LamRR_BAMort = Int((LamRR_HostBA * 3) * (LamRisk_Final * .01))
LamRR_%Mort = Int((LamRR_BAMort / LamRR_HostBA) * 100)

## Preliminary Results and Discussion

The initial Laminated Root Disease Risk Model outputs for Oregon and Washington using the new variables averaged 12% mortality in host species on acres with modeled risk, which is consistent with mortality rates reported by Hansen and Goheen in 2000. Spatial patterns of predicted future mortality in host species around confirmed laminated root rot centers showed the highest expected losses at sites immediately adjacent to known root disease centers (Figure 4). The spatial resolution of model outputs provides a finer scale map product than NIDRM that has a pixel size of ~14 acres. Using finer scale, regionally-derived vegetation/host data could also improve these risk models compared to vegetation datasets used in NIDRM. Utilizing imputed vegetation data where the FIA intensified plot grid exists could be explored more in different parts of the U.S. Additional work is needed to assess the accuracy of these model outputs and to refine the models using the most recent GNN data through 2017. These approaches evaluating current conditions could also be incorporated into future bioclimatic modeling efforts (Klopfenstein et al. 2009).

Potential uses of Root Disease Risk Models include:

- Input to Restoration Project planning
- Improved detection of root diseases, target high risk areas for root disease field surveys for prioritizing treatments and planting tolerant/resistant species
- Forest health monitoring and to set expectations for forest change and wildlife habitat in National Forest Plan revisions
- Incorporate into departure analyses, watershed health assessments, fire & fuels modeling, climate change vulnerability and other broad scale assessments

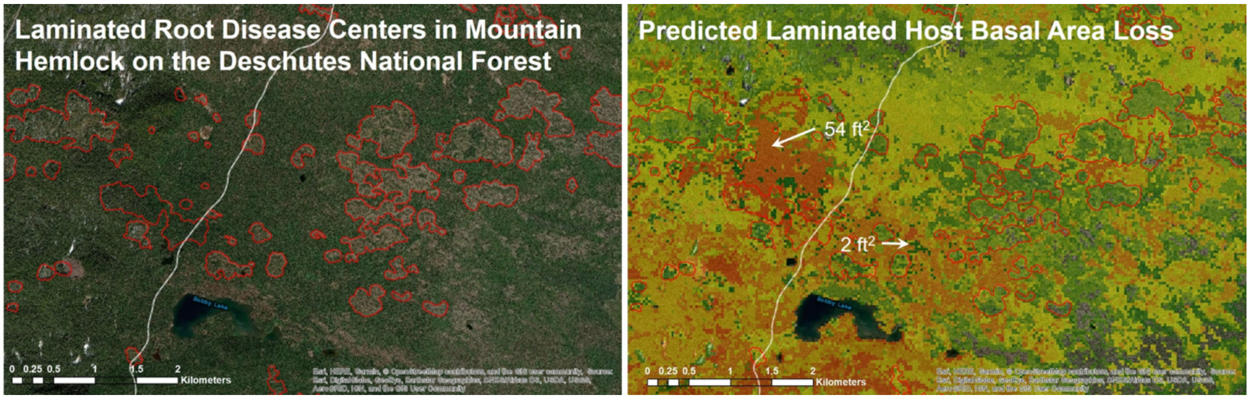


***Figure 4:*** *Spatial pattern of predicted loss in host species basal area (ft²/acre) in 15 years using the Laminated Root Disease Model in mountain hemlock around documented laminated root disease centers near Bobby Lake (just east of Waldo Lake), Oregon Central Cascades. On the right, orange-to-red equate to greater basal area losses and green-to-yellow equate to lower predicted losses.*

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Washington's 20 Year
Forest Health Strategic Plan:
Preparing Landscapes for the Future.

# IMPROVED STRESS DETECTION IN TREES USING NASA GODDARD'S LIDAR, HYPERSPECTRAL AND THERMAL IMAGING SYSTEM (G-LIHT)

**Ryan P. Hanavan[1], Bruce D. Cook[2], Lawrence A. Corp[2], Brent W. Oblinger[3],**
**Mike Simpson[3], and Robbie W. Flowers[3]**

## Summary

The Goddard LiDAR, Hyperspectral and Thermal imaging system (G-LiHT) provides data fusion opportunities to increase accuracy and early detection of incipient infestations of forest insects and diseases (e.g., Pontius et al. 2017, Meng et al. 2018). Forest Health Protection (FHP) Annual Insect and Disease Surveys (IDS, also known as aerial detection surveys) created a unique opportunity to develop an enhanced survey project between the U.S. Forest Service and NASA. The G-LiHT captures both upwelling and downwelling irradiance and contains both profiling and scanning LiDAR instruments. The G-LiHT is easily mounted in planes equipped with camera ports conducting IDS missions.

We selected an Area of Interest (AOI) in southcentral Oregon with known Armillaria root disease in white and Shasta red firs. Our goal was then to identify the most accurate approach to species-level mapping across a range of decline conditions related to Armillaria root disease (live and dead standing, dead with no foliage attached, recently-killed with discolored foliage attached, live infected trees with chlorotic or fading foliage, and live, healthy trees). We then used object-based segmentation techniques, spectral mixture analysis, and combinations of Vegetation Indices along with hyperspectral bands in unmixing calibration procedures to map root disease centers and identify stress conditions within the AOI. Additional work is ongoing to develop methods for utilizing LiDAR data (e.g., intensity of point returns) to quantify fine branch dieback and other conditions that will improve our modeling efforts. More study areas are planned for 2019 where airborne and field/ground data collection would occur to continue evaluating approaches to detecting forest root diseases using the fusion of aerial imagery and LiDAR data.

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# CONTRIBUTED POSTERS

# CHARACTERIZATION OF THE PINE WILT DISEASE PATHOSYSTEM IN THE FRONT RANGE REGION OF COLORADO

**David Atkins[1], Jane Stewart[2], and Seth Davis[1]**

The pinewood nematode (*Bursaphelenchus xylophilus;* PWN) causes a lethal wilting disease in susceptible pine trees. This pathogen has recently been discovered in Colorado in both non-native (2006) and native (2016) pines. PWN is typically vectored by sawyer beetles (*Monochamus* spp.), of which there are two species found in Colorado *(M. clamator* and *M. scutellatus*). Understanding the vector will be critical in preventing the spread of PWN. The goal of the study was to determine which *Monochamus* species vector PWN, model vector flight phenology, and to estimate factors that may affect pathogen load within the vectors. Here, we report findings from trapping efforts in 32 sites with predominantly ponderosa pine (*Pinus ponderosa*) cover along the Front Range area. These findings include documentation of PWN in both species of sawyer beetle, a vector-pathogen association (*M. clamator*-PWN) previously unreported in the U.S., as well as flight phenology models coupled with robust evidence in support of preferential colonization of recently burned areas for both species.

*Monochamus clamator* was found to have an infection rate of 2.81% (51/1814), while *M. scutellatus* was found to have an infection rate of 4.4% (16/364). The infection rate was similar between species (Chi-squared p-value = 0.17). Flight phenology was modeled as a logistic regression with cumulative proportion of beetle capture for each species as the response and ordinal day as the predictor. Vector flight appears to be synchronous between species. Results of a multiple-regression analysis including a variety of landscape variables indicated distance to fire was a significant predictor for *M. scutellatus* abundance at each site (p=0.042, $R^2$=0.13, df=1,30), while the model for *M. clamator* also included distance to the eastern forest edge and an interaction between ‘distance to city boundary’ and ‘average % canopy cover within 250m’ (p=0.003, $R^2$=0.48, df=5,26). Models were selected via minimization of AICc using train-test data subsets. These findings will help management efforts target the highest abundance of vectors both spatially and temporally.

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# INSIGHTS INTO THE EPIDEMIOLOGY AND DISPERSAL CAPABILITIES OF *LEPTOGRAPHIUM WAGENERI* VAR. *PSEUDOTSUGAE* WITHIN AND BETWEEN YOUNG DOUGLAS-FIR STANDS IN WESTERN OREGON

**Patrick I. Bennett[1,2*] and Jared M. LeBoldus[2,3]**

## Introduction

Black stain root disease (BSRD) is a vascular wilt that causes mortality in several conifer species (Wagener & Mielke 1961, Harrington & Cobb 1987). It is caused by the fungus *Leptographium wageneri*, which can spread via root contact and is also vectored by insects (Witcosky & Hansen 1985, Witcosky et al. 1986). Douglas-fir (*Pseudotsuga menziesii*) is host to a specific variety of the fungus, *L. wageneri* var. *pseudotsugae* (Harrington & Cobb 1987). The fungus is vectored by two species of weevil (*Pissodes fasciatus*, *Steremnius carinatus*) and the Douglas-fir root beetle (*Hylastes nigrinus*) (Witcosky & Hansen 1985, Witcosky et al. 1986). The incidence and severity of BSRD in young Douglas-fir plantations in the Pacific Northwest has intensified in recent years. This disease is emerging as a serious threat to the health and productivity of young trees in managed forest stands in Oregon. The population structure of the BSRD fungus as it relates to epidemiology has not been investigated previously. It is not currently known whether local or long-distance dispersal is more important for the establishment of new BSRD infections, and the relative genetic diversity of *L. wageneri* var. *pseudotsugae* within and between stands has not been described. This study was designed to address these gaps in knowledge and may provide insights into the emergence and spread of BSRD. The specific objectives were to: 1) sequence genomes of *L. wageneri* var. *pseudotsugae* isolates collected in western Oregon Douglas-fir plantations; 2) identify single nucleotide polymorphisms (SNP) to assign genotypes; and 3) compare population structure and diversity among plantations, and among stands within plantations, to make inferences about epidemiological characteristics such as dispersal and colonization.

## Methods

Wood samples showing characteristic staining were collected from the root crowns of 107 symptomatic *P. menziesii* trees in 11 stands across three spatially disjunct timber plantations in western Oregon (Figure 1). Additionaly, six isolates of *L. wageneri* var. *wageneri* were collected from two single-leaf pinyon pine (*Pinus monophylla*) stands in the San Bernardino National Forest in southern California. All field samples were kept on ice and returned promptly to the lab, and isolations were performed within 14 days following collection. Stained wood pieces were surface sterilized and plated on a selective medium consisting of 1.5% malt agar supplemented with 100 ppm streptomycin and 200 ppm cycloheximide (CSMA) (Harrington 1992, Jacobs & Wingfield 2001). Cultures were incubated for 2-4 weeks. Fungi displaying morphological characteristics consistent with *L. wageneri* were isolated onto 2% malt extract agar (MEA) and incubated for an additional 2-4 weeks. Mycelium from the MEA plates was then transferred to 2% liquid malt extract broth and grown for 2-4 weeks prior to the extraction and purification of genomic DNA.

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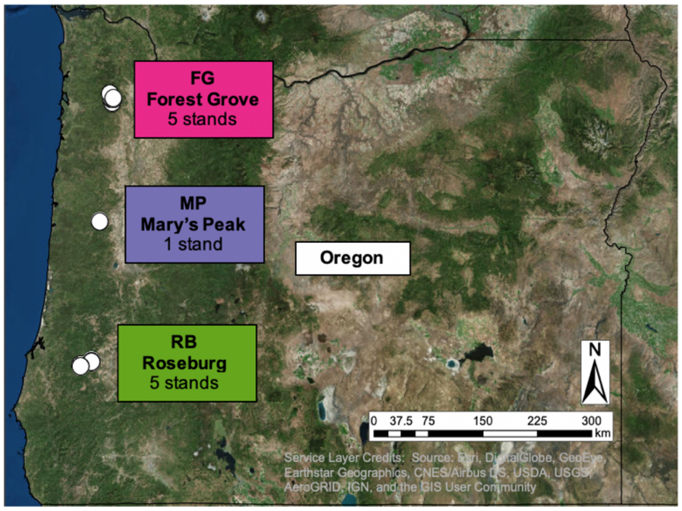

***Figure 1:*** *Map showing the locations of the three Douglas-fir plantations in western Oregon (FG, MP, RB) from which* L. wageneri *var.* pseudotsugae *isolates were collected. A total of 47 isolates were collected from five stands at the FG plantation, 10 isolates were collected from one stand at the MP plantation, and 50 isolates were collected from five stands at the RB plantation.*

Whole genome sequencing was performed for all isolates with an Illumina HiSeq 3000 with 150 bp paired-end reads. All DNA extractions, library preparation, and Illumina sequencing were performed by the Center for Genome Research and Biocomputing at Oregon State University in Corvallis, Oregon. The Illumina reads were aligned to a reference genome of *L. wageneri* var. *pseudotsugae* type strain CMW154, and SNPs were identified to assign individual genotypes. The R statistical computing platform (R Core Team 2018) was used along with the package vcfR (Knaus & Grunwald 2017) for quality filtering. The filtering protocol masked all SNP variants with < 10x sequencing coverage, all samples with > 55% missing data, and all variants with > 20% missing data. A series of population genetic analyses were performed in R with *poppr 2.8.1* (Kamvar et al. 2014, Kamvar et al. 2015) and *adegenet* (Jombart 2008, Jombart et al. 2010).

## Results

After quality filtering, the final dataset included 112,577 genome-wide SNPs. The branch topology and support values in the neighbor-joining dendrogram suggested that the two *L. wageneri* varieties are highly differentiated and that no gene flow occurs between them (Figure 2). The *L. wageneri* var. *pseudotsugae* isolates collected from three plantations in western Oregon represented two distinct genetic clusters. One cluster contained only isolates collected at the FG plantation, while the other consisted of a mixture of isolates from the three plantations (Figure 2).

The scatter plot from a discriminant analysis of principal components (DAPC) (Jombart et al. 2010) revealed some overlap between the clusters representing the three plantations. This indicates that some of the isolates from the three plantations have similar genotypes (Figure 3A). The DAPC genotype composition plot (Figure 3B) indicated that the genotypes of isolates collected in the MP and FG plantations have a high probability of membership in the cluster represented by the RB

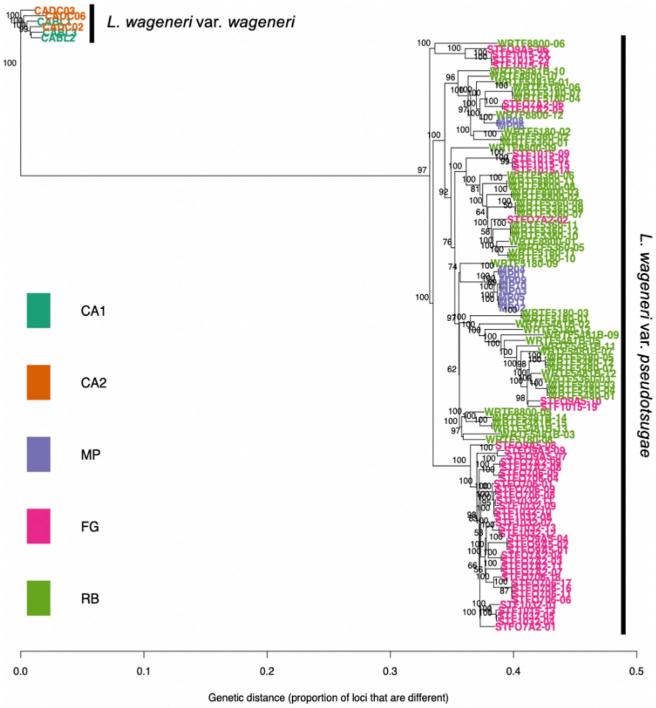

***Figure 2:*** *Genetic distance (neighbor-joining) tree constructed with 112,577 genome-wide SNPs from 107 isolates of* L. wageneri *var.* pseudotsugae *and six isolates of* L. wageneri *var.* wageneri. *Branch support values were calculated from 100 bootstrap replicate trees.*

isolate genotypes. This suggested that the isolates collected from the MP and FG plantations are more similar to those collected in the RB plantation than they are to the other isolates collected in the MP and FG plantations. These results were supported by genetic differentiation ($G'_{ST}$) (Hedrick 2005) values calculated pairwise among each of the plantations. The isolates from the MP and FG plantations were more strongly differentiated than the isolates from the FG and RB plantations or from the MP and RB plantations (Table 1).

An analysis of molecular variance (AMOVA) assessed the spatial distribution of genetic diversity within and between plantations. The results were consistent with the other analyses in that they indicated that most of the genetic variation (53.37%) occurred within stands (i.e. stands are a diverse and heterogeneous mixture of isolates) (Table 2). The remaining genetic variation occurred between stands within plantations (19.84%) and between plantations (26.79%) (Table 2).

***Table 1:*** *A standardized genetic differentiation measure ($G'_{ST}$) calculated pairwise for the three plantations sampled. This statistic is equal to 0 when all alleles are shared among populations and 1 when no alleles are shared.*

| | MP | FG | RB |
|---|---|---|---|
| **MP** | 0 | - | - |
| **FG** | 0.228 | 0 | - |
| **RB** | 0.119 | 0.156 | 0 |

***Table 2:*** *Analysis of molecular variance (AMOVA) table showing the partitioning of genetic variance among levels of a population hierarchy. The phi statistic (φ) is an estimate of genetic differentiation. The P-value for each phi statistic was calculated from 999 permutations of a randomization test.*

| Hierarchical Level | Variance (%) | ϕ | P * |
|---|---|---|---|
| $\phi_{ST}$ (between plantations) | 26.787 | 0.268 | 0.002 |
| $\phi_{SS}$ (between stands within plantations) | 19.842 | 0.271 | 0.001 |
| $\phi_{IS}$ (within stands) | 53.370 | 0.467 | 0.002 |

## Discussion

This study examined genetic differentiation between two of the three host-specific *L. wageneri* varieties and compared genetic structure and variation within and among 11 stands in three separate Douglas-fir plantations in western Oregon (Figure 1). The SNP genotypes representing isolates from the varieties of *L. wageneri* occurring on single-leaf pinyon pine (*P. monophylla*) and Douglas-fir (*Pseudotsuga menziesii*) were highly differentiated due to a lack of gene flow (Figure 2) reflecting their evolutionary divergence. Whether these groups represent separate species or should be maintained as varieties of the same species should be investigated further with phylogenetic analyses.

Although sexual reproduction has not been observed in the Douglas-fir variety of *L. wageneri*, there was more genetic variation present in populations of *L. wageneri* var. *pseudotsugae* than would be expected from asexual reproduction alone. Other sources of variation could include migration, diversifying selection, genetic drift, or random mutation. The three plantations sampled in western Oregon were approximately 300 km (186 mi) apart, but some of the isolates collected at each of the plantations had similar genotypes suggesting that they were closely related. This indicates migration as a source of variation within plantations. The co-occurrence of isolates from all three plantations in a single genetic cluster in the neighbor-joining dendrogram (Figure 2), overlap between DAPC clusters (Figure 3A), posterior membership probabilities (Figure 3B), and moderate genetic differentiation among some of the plantations (Tables 1 and 2) are all consistent with gene flow occurring between plantations. Another explanation could be that the *L. wageneri* populations collected in the separate plantations came from a common source population.

Natural dispersal by insect vectors over the 300 km distance between the FG and RB plantations is unlikely. The weevil *S. carinatus* is flightless, while the Douglas-fir root beetle (*H. nigrinus*) and *P. fasciatus* are capable of flight (Jacobi 1992), but their dispersal capabilities are not known. It is more likely that the similarity of genotypes across western Oregon plantations is due to the fact that the Douglas-fir host has a continuous distribution in this region, and thus spatially disjunct timber plantations may be interconnected by shorter-distance insect flights in a sort of “stepping-stone” fashion (Linde et al. 2002). Overall, the evidence suggests that the insect-mediated dispersal of *L. wageneri* between stands has a greater influence on the genetic structure of its populations than local vegetative spread.

The apparent migration among plantations could also result from human-mediated migration of *L. wageneri* or its insect vectors. It is not known whether infested trees, logs, soil, or equipment may be moving between these plantations or whether these plantations share a common source such as a nursery that may be infested with *L. wageneri* or its vectors. These possibilities should be investigated in future studies.

Migration between stands within plantations has resulted in high stand-level genetic variation (Table 2). The variation observed within stands is not consistent with a single infection and subsequent vegetative spread via root contact, but rather suggests that an infected stand is a heterogeneous mix of isolates that are often distantly related. This is most likely the result of multiple independent infections occurring in each stand, and reflects the importance of insect-mediated dispersal relative to spread via root contact. This might be expected in younger Douglas-fir stands where adjacent trees are spaced far enough apart such that their root systems have not yet come into contact. The stand-level diversity also reflects the diversity of *L. wageneri* present in surrounding stands and in the insect vector populations that serve as the sources of inoculum for each BSRD infection. Future studies of *L. wageneri* diversity and epidemiology could be facilitated by trapping insect vectors and analyzing the genetic variation of *L. wageneri* isolates on individual insects.

It is important to consider the fact that insect vectors regularly transport *L. wageneri* between stands when planning timber harvest activities. For instance, it is known that activities such as thinning as well as soil compaction resulting from roads and skid trails attract the insects that vector *L. wageneri* (Witcosky et al. 1986, Hessburg et al. 2001, Kelsey & Joseph 1998). Considering the fact that the vectoring of *L. wageneri* from outside a stand seems to be much more important than vegetative spread within young Douglas-fir stands, managers may choose to keep these impacts to a minimum to avoid attracting vectors from nearby BSRD infection centers. Given an estimate of the dispersal capabilities of *L. wageneri* vectors, managers may also be able to better locate timber harvest and management activities away from stands that are known to be infested with *L. wageneri* and its vectors.

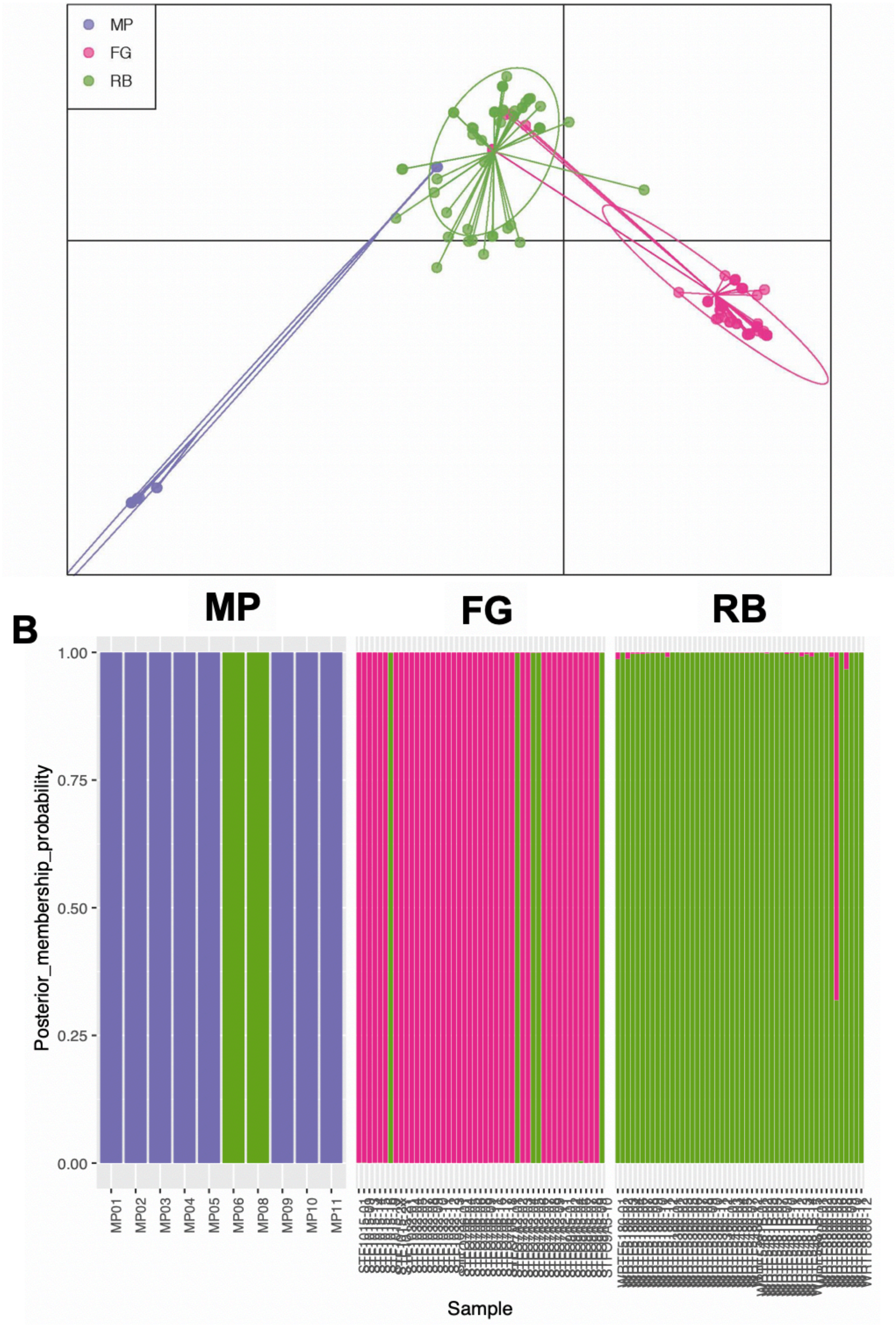

***Figure 3:*** *Discriminant analysis of principal components (DAPC).* ***A)*** *Scatter plot showing clustering among isolates of* L. wageneri *var.* pseudotsugae *collected from three Douglas-fir plantations in western Oregon.* ***B)*** *Genotype composition plot showing the posterior membership probability of each isolate collected in the three plantations.*

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# MOUNTAIN PINE BEETLE-CAUSED TREE MORTALITY HAS NO APPARENT INFLUENCE ON *ARMILLARIA* IN PONDEROSA PINE

**James T. Blodgett[1] and Kurt K. Allen[1]**

## Introduction

Studies have suggested pine infected by Armillaria root disease may be more susceptible to endemic mountain pine beetle (MPB; *Dendroctonus ponderosae*) attack (Tkacz & Schmitz 1986, Nebeker et al. 1995). However, the influence of mountain pine beetle mortality on *Armillaria* has never been examined. Tree mortality caused by fire (Blodgett & Lundquist 2004, 2008) and timber harvesting (Filip et al. 2010, Roth et al. 1980; Roth et al. 2000, Shaw 1980) can result in increased frequency of *Armillaria solidipes* (*A. ostoyae*), an important root pathogen of conifers. The objective of this study was to determine if tree mortality caused by mountain pine beetle causes similar increases in *A. solidipes* in ponderosa pine (*Pinus ponderosa*) stands.

## Methods

Three study sites were established in the Black Hills National Forest of South Dakota (Figure 1). Study sites were chosen based on the presence of heavy MPB activity. In 2015, study site 1, plots were established in areas of a stand with no (Figure 2A) or current-year (Figure 2B) MPB infestations, or in areas where MPB infestations occurred 3 years (Figure 2C) or 6 years (Figure 2D) prior (four treatments). In 2016 the experiment was repeated at site 1 using the same plots. In 2017, two additional study sites were selected in the forest (sites 2 and 3). At these sites, plots were established in areas of the stands with no MPB infestations, current-year MPB infestations, or where MPB infestations occurred 4 years prior (three treatments). Five plots were established per treatment at each of the three study sites.

*Armillaria* baits (stem-sections) were cut from live ponderosa pine stems (at least 0.5 m above the soil) in June. They measured 8 cm long by 2.5 to 4 cm in diameter and were maintained at 4° C until use in the experiment (within 2 days). The stem-sections were buried flush with the top of the mineral soil layer and covered with the organic soil layer. Twenty stem-sections were used per plot (Figure 3). Approximately 1 year after stem-sections were buried, they were excavated, and the percentage of stem-sections colonized by *Armillaria* was visually evaluated (Figure 4).

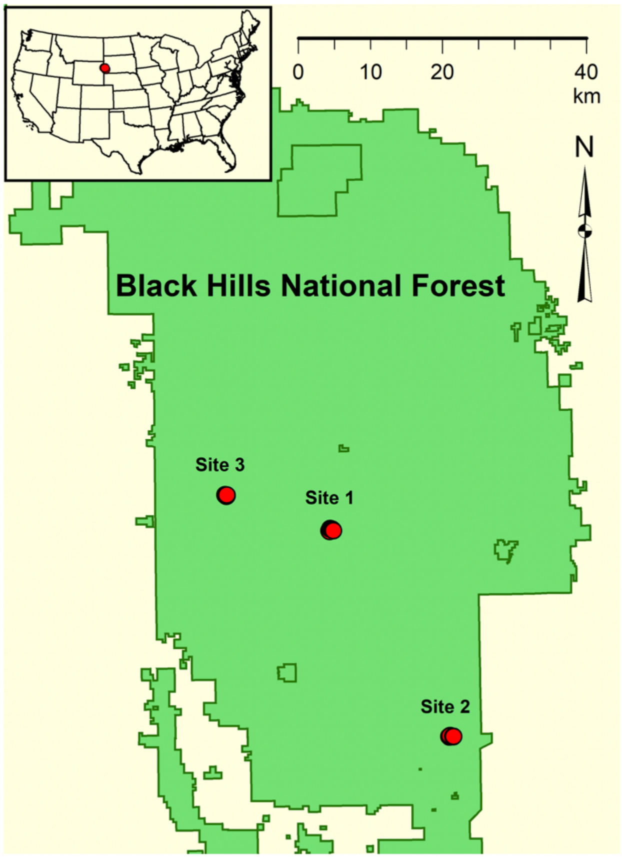

***Figure 1:*** *Location of the three study sites in the Black Hills National Forest of South Dakota. The study consisted of twenty plots at site 1 (used in 2016 and 2017) and fifteen plots each at site 2 and site 3 (used in 2018).*

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[1] *USDA Forest Service, Rocky Mountain Region, Forest Health Protection, Rapid City, SD, USA*

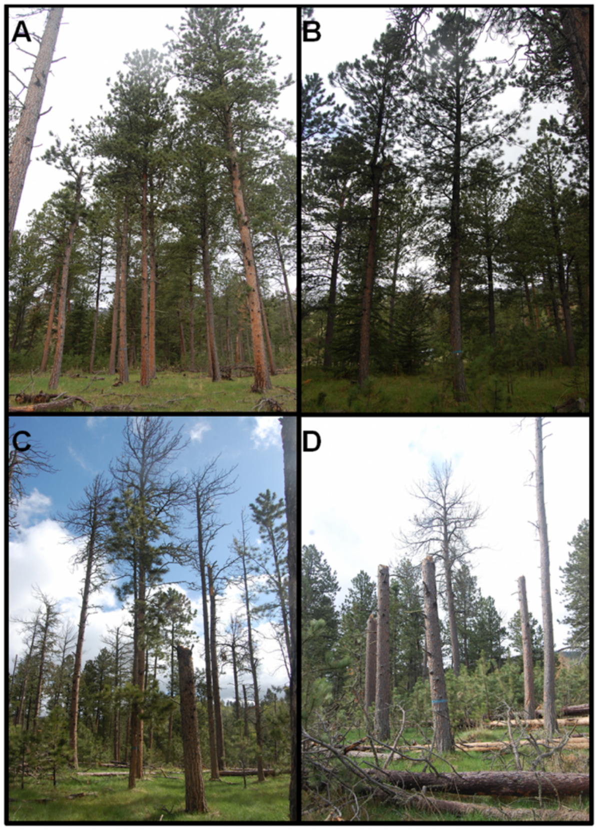

***Figure 2:*** *Examples of treatments included in the study. Treatments represent different infestation times and a MPB uninfested control. At study site 1, plots were established in areas of a stand with no (**A**) or current-year (**B**) MPB infestations, or in areas where MPB infestations occurred 3 (**C**) or 6 (**D**) years prior; or 4 years prior for study sites 2 and 3 (not shown).*

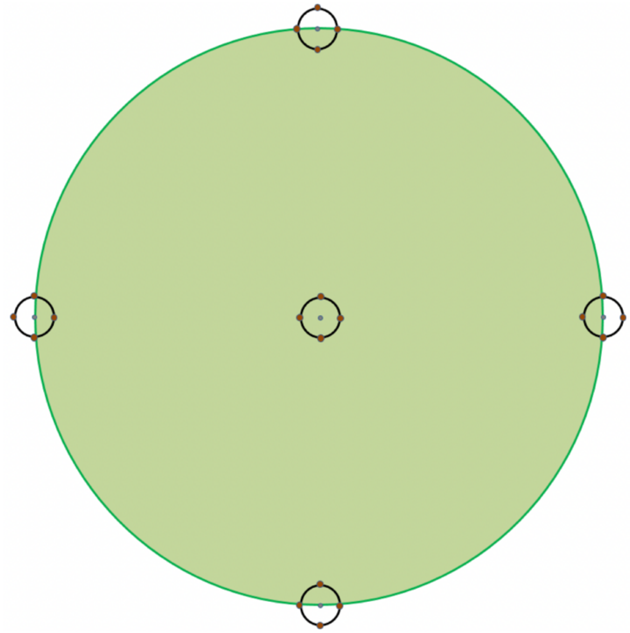

***Figure 3:*** *The large green circle represents a 0.02 ha plot; black circles represent 30.5 cm radius around a metal stake; brown dots represents stem-sections. There were 20 stem-sections per plot (4 stem-sections 0.3 m from plot center and from the 4 cardinal directions). Five plots were used per treatment, or 100 stem-sections per treatment at each of the three study sites.*

**Results**

Trees averaged 28 ± 5 cm DBH, and plots averaged 83 ± 36 trees per ha. No recent mortality occurred in uninfested plots, but 78% of the trees were recently killed in MPB-infested plots.

*Armillaria* colonization of stem-section bait did not increase with MPB activity. For 2016 and 2017 (site 1; combined since differences were not significant between years; $P = 0.892$), differences in the proportions of stem-sections colonized by *A. solidipes* were not significant among MPB treatments (Figure 5; $P = 0.989$). In 2018 (sites 2 and 3 combined), differences were again not significant among MPB treatments ($P = 0.110$). The percentage of colonized stem-section baits did differ among study sites ($P < 0.001$); the average percentages were 57% (site 1; 2016), 57% (site 1; 2017), 39% (site 2; 2018), and 48% (site 3; 2018).

***Figure 4:*** *A stem-section colonized by* Armillaria solidipes *(left), and a non-colonized stem-section (right).*

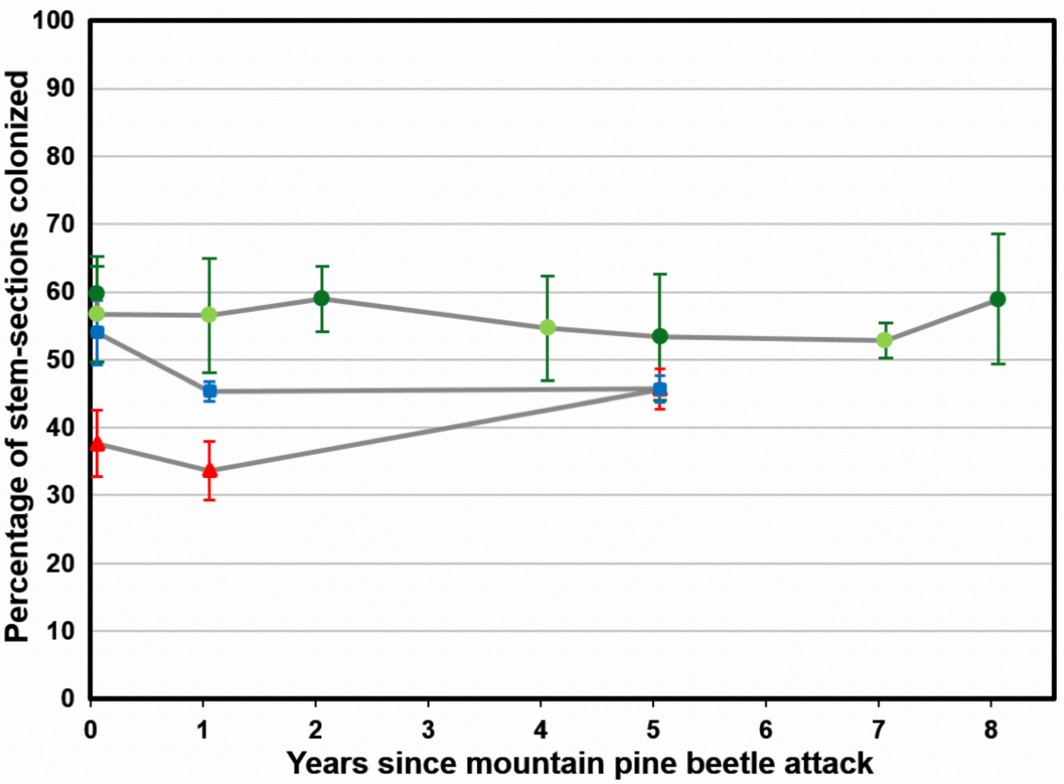

***Figure 5:*** *The percentage of colonized stem-sections were used to compare treatments in 2016 and 2017 at study site 1 (● and ●, respectively); and in 2018 at study sites 2 and 3 (■ and ▲, respectively). Each point in graph represents 100 stem-sections per treatment (1,400 total stem-sections).*

## Discussion

Results indicate MPB-caused mortality does not influence the frequency of *A. solidipes* colonization of buried stem-section baits. Using similar *Armillaria* bait methods, fire-caused tree mortality resulted in an increase in stem-section bait colonization (Blodgett & Lundquist 2008). The current finding suggests different ecological processes occur in root systems of trees killed by MPB compared with trees killed by fire and timber harvesting. A potential explanation might be that available nutrients (*e.g.*, sugars and starches) differ within roots of MPB-killed trees.

The lack of a measurable *Armillaria* response in relation to MPB-attack might be caused by indirect (*i.e.*, between stems and roots) competition for available resources. Bark beetles, the blue-stain fungus and other fungi introduced by the beetles, and the trees (through maintenance, growth, defense, etc.) utilize the carbohydrates as trees slowly die. Compared to the sudden mortality resulting from fire and timber harvesting, tree mortality occurs more slowly following MPB attack, which could result in limited carbohydrate availability in roots.

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# TRANSFORMATION OF THE TREE: DWARF MISTLETOE IMPACTS ON CROWN STRUCTURE AND SAPWOOD AREA OF WESTERN HEMLOCK

**Stephen Calkins[1] and David Shaw[1]**

Western hemlock dwarf mistletoe (*Arceuthobium tsu*gense ssp. *tsugense*) is an arboreal, hemiparasitic plant that parasitizes western hemlocks (*Tsuga heterophylla*). Dwarf mistletoe generates host responses in infected trees that can drastically change the structure of the tree crown. Dwarf mistletoe also sharply reduces host's total water use and carbon accumulation through reduction of water use efficiency. These changes to the tree result in unique habitat structure, changed fire dynamics, and more severe drought impacts. Comprehensive studies on individual tree transformations are limited. In this study we employ crown mapping to address this knowledge gap. Crown mapping involves climbing and intensively measuring tree and branch structures to produce detailed models of tree crowns; this will be the first set of mapped mature western hemlock crowns infected with dwarf mistletoe.

**Our study's objectives are to:**

1) Examine how the western hemlock tree crown structure changes across an infection severity gradient at the branch and crown level;
2) Examine changes in diameter growth and sapwood area of the tree stem across an infection severity gradient. Sixteen old growth hemlocks were mapped at the HJ Andrews Experimental Forest, Blue River, OR.

Each tree's branches and stem were measured and up to 14 cores were taken at seven locations vertically to develop a profile of stem sapwood area. To explore different measures of infection severity, three measures were developed and used to individually model over 25 different tree-level responses. Severity was defined in the models as the total infection incidence, proportion of all live branches infected, and proportion of all live, infected branches with 30 percent or more foliage affected by infection. Transformation of the tree crown by intensification of dwarf mistletoe had a profound influence on foliage, branches, and crown volume, although relative sapwood area was unaffected.

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Structure, Morphology, and Severity

# TOOLS FOR EARLY DETECTION AND MONITORING OF THE KOA WILT PATHOGEN (*FUSARIUM OXYSPORUM* F. SP. *KOAE*) IN HAWAIʻI

**John T. Dobbs[1], Mee-Sook Kim[2], Nicklos S. Dudley[3], Tyler C. Jones[3], Aileen Yeh[3], R. Kasten Dumroese[4], Philip G. Cannon[5], Robert D. Hauff[6], Ned B. Klopfenstein[4], and Jane E. Stewart[1]**

Koa (*Acacia koa*), the second most common endemic tree and having considerable ecological, economic, and cultural importance to Hawaiʻi, is under threat from koa wilt disease (caused by *Fusarium oxysporum* f. sp. *koae*; *Fo koae*), particularly in low- to mid-elevation forests. *Fusarium oxysporum* is a cosmopolitan vascular wilt pathogen of diverse plant hosts. Morphological identification methods for discerning pathogenic and non-pathogenic *F. oxysporum* strains are unreliable, but after screening for pathogenicity, our genetic analyses of 24 *F. oxysporum* isolates collected from koa showed a clear and well-supported clade of pathogenic *Fo koae* isolates. Because of these results, our objectives were to 1) conduct and analyze whole genome sequencing of one highly-virulent *Fo koae* isolate and one non-pathogenic *F. oxysporum* isolate; 2) identify virulence-associated genes that are putatively important for disease development in the host; and 3) use the identified differences to develop a *Fo koae*-specific PCR primer. Using whole-genome sequencing of one highly virulent *Fo koae* isolate and one non-pathogenic *F. oxysporum* isolate, unique sequences were identified that may provide distinguishing features associated with koa wilt pathogens. Subsequently, primers were developed based on these unique sequences to identify pathogenic strains of *Fo koae* and were verified on pathogenic (12) and non-pathogenic (5) characterized isolates of *F. oxysporum* and isolates of *F. commune* (2) and *F. proliferatum* (1). These other *Fusarium* spp. were included because of their morphological similarity to *F. oxysporum* and because they have been previously isolated from koa. Koa breeding programs aid restoration efforts that remediate losses of koa in lowland forests and timber stands. To develop the strongest resistance to koa wilt disease, these programs require highly virulent strains of *Fo koae*. The developed pathogen-specific primer offers potential to improve koa restoration and resistance-breeding programs by introducing a faster, more reliable screening method for identifying highly virulent strains of *Fo koae*.

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Acacia koa
Endemic tree species to Hawai'i
>2nd most abundant tree
Very valuable wood
>Koa Industry: valued at $30 million
Fusarium oxysporum
Important vascular wilt pathogen on many plant species worldwide
Causes significant dieback in koa
Can be pathogenic or saprophytic without discernable morphological differences

# HEALTH AND ABUNDANCE OF SOUTHWESTERN WHITE PINE AND LIMBER PINE IN SOUTHERN COLORADO AND NORTHERN NEW MEXICO

**Megan M. Dudley[1], Kristy L. Duran[1], and Kelly S. Burns[2]**

Little data is available on the health and abundance of white pine populations in southern Colorado and northern New Mexico. This study characterizes stand structure, composition, and forest health conditions in these understudied ecosystems. Since July 2018, we have been conducting a detailed survey of five-needle pine populations on Forest Service lands in southern Colorado and northern New Mexico to determine the general health of these understudied ecosystems in the project area, and to specifically survey for White Pine Blister Rust (WPBR). From July-September of 2018, we installed 25 survey plots on the Rio Grande National Forest, three survey plots on private land east of the San Luis Valley, and one plot on the Great Sand Dunes National Park. We WPBR at a new location (on private land), in the Indian Creek area, roughly 42 km south-east of the previously-described infection site at Mosca Pass. We detected other rust species on *Ribes* sp. at one additional location on the Rio Grande National Forest. Rust samples were submitted to the Stewart lab at Colorado State University for identification. These have tentatively been identified as *Cronartium ribicola* and *Puccinia caricis.* Survey work will continue through the 2019 field season, with additional plots installed on the Rio Grande, Carson, San Juan, and Gunnison National Forests.

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*[1]Adams State University, Alamosa, CO. [2]USFS Forest Health Protection*

# TESTING RESISTANCE OF ROOTSTOCK MATERIALS FOR MANAGING ARMILLARIA ROOT DISEASE IN PEACH ORCHARDS OF MÉXICO

**Rubén Damián Elías-Román[1], Guillermo Calderón-Zavala[2], Rafael Guzmán-Mendoza[1], Moises Vallejo[3], Ned B. Klopfenstein[4], and J. Antonio Mora-Aguilera[5]**

## Abstract

*Armillaria mexicana* and/or *A. mellea* cause Armillaria root disease that results in mortality of peach (*Prunus persica*) trees in orchards of the Mexican sub-tropics. Resistance/susceptibility reactions to *A. mexicana* and *A. mellea* were evaluated for three *Prunus* rootstocks under greenhouse and field conditions. For greenhouse tests, independent inoculations of *A. mexicana* and *A. mellea* were conducted on 14 trees for each of three rootstocks: *P. persica* × *P. davidiana* 'Nemaguard', *P. persica* landrace 'Criollos of La Goleta' genotypes, and *P. salicina* Japanese plum 'Mondragon', with four non-inoculated trees serving as controls for each rootstock. For greenhouse inoculations with *A. mexicana*, 'Criollos of La Goleta' and 'Nemaguard' rootstocks showed the highest incidence of infected roots (21.6% and 24.6%, respectively). In contrast, 'Mondragon' showed the lowest (2.5%) incidence of infected roots. Although 'Criollos of La Goleta' produced the highest root dry weights, root volumes, and plant dry weights, its root systems contained abundant mycelial infections. Under greenhouse conditions, an overall lower (17.5%) incidence of root infection was observed after inoculations with *A. mellea*. Inoculations with *A. mexicana* were performed in a commercial orchard that was naturally infested with *A. mexicana* using 21 plants of 'Mondragon' (cutting-derived), 'Criollos of La Goleta', and *P. mume* 'Japanese apricot'. The lowest susceptibility to *A. mexicana* was exhibited by 'Mondragon' rootstock. Management of Armillaria root disease in peach orchards of Mexico depends on identifying *A. mexicana*-resistant rootstock, such as 'Mondragon' (Elías-Román et al. 2019).

## Introduction

Armillaria root disease causes wide-spread and severe damage/mortality within peach (*Prunus persica*) orchards in subtropical Central México (e.g., Morelos, Michoacán, and State of México), which is a primary region for peach production (Coria-Avalos et al. 2005, Alvarado-Rosales 2007, Fucikovsky 2009, Elías-Román et al. 2013, Rivas-Valencia et al. 2017, Elías-Román et al. 2018). In this region of México, Armillaria root disease of peach is caused by *A. mellea*, *A. gallica*, and *A. mexicana*, a recently described species (Elías-Román et al. 2018). Management strategies are critically needed to reduce *Armillaria*-caused mortality of peach trees to promote sustainability and production for commercial orchards within the prominent peach-production areas of México. The objective of this study was to evaluate the resistance/susceptibility of four *Prunus* rootstocks to infection by *A. mexicana* and/or *A. mellea* under greenhouse and/or field conditions.

## Materials and Methods

Rootstock resistance to Amillaria root disease were evaluated under greenhouse and field conditions (Figures 1 and 2).

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Rootstocks – Four *Prunus* rootstocks for peach were tested:

1) *P. persica* x *P. davidiana* 'Nemaguard' (greenhouse test only);
2) *P. persica* landrace 'Criollos of La Goleta' genotypes;
3) *P. mume* seedlings, Japanese apricot (field test only); and
4) *P. salicina*, Japanese plum 'Mondragon'.

Inoculum – Seeds (acorns) of oak (*Quercus* sp.) were used to increase *Armillaria* inoculum, according to the procedure of Beckman & Pusey (2001) (Figure 1). *Armillaria mexicana* (isolate MEX85) and *A. mellea* (isolate MEX100) were used for greenhouse inoculations; whereas MEX85 isolate was used for field inoculations within a peach orchard that was heavily infested with *A. mexicana* (Figures 1 and 2).

Variables evaluated – Infected root incidence % (IRI), Dead root incidence % (DRI), Root dry weight (RDW), Root length of lateral roots (RL), Root volume (RV), Plant dry weight (PDW), Disease severity (0 – 4 scale, field test only, Figure 3), and Weighted average severity (WAS) in the field, according to the formula of Guzman-Plazola et al. (2004).

Experimental design and statistical analysis – In the greenhouse, a split plot with completely randomized design was used with a factorial arrangement. Incidence value percentages were transformed into their arcsin values using arcsin√x/100. The retransformed means to original units are shown in Table 1. The ANOVA was performed with the GLM procedure and the comparison of means with LSmeans (SAS®, 2011). In the field, trees were established in a randomized block design, with seven replications. The severity values of field experiment were analyzed using the Friedman test in InfoStat (2008).

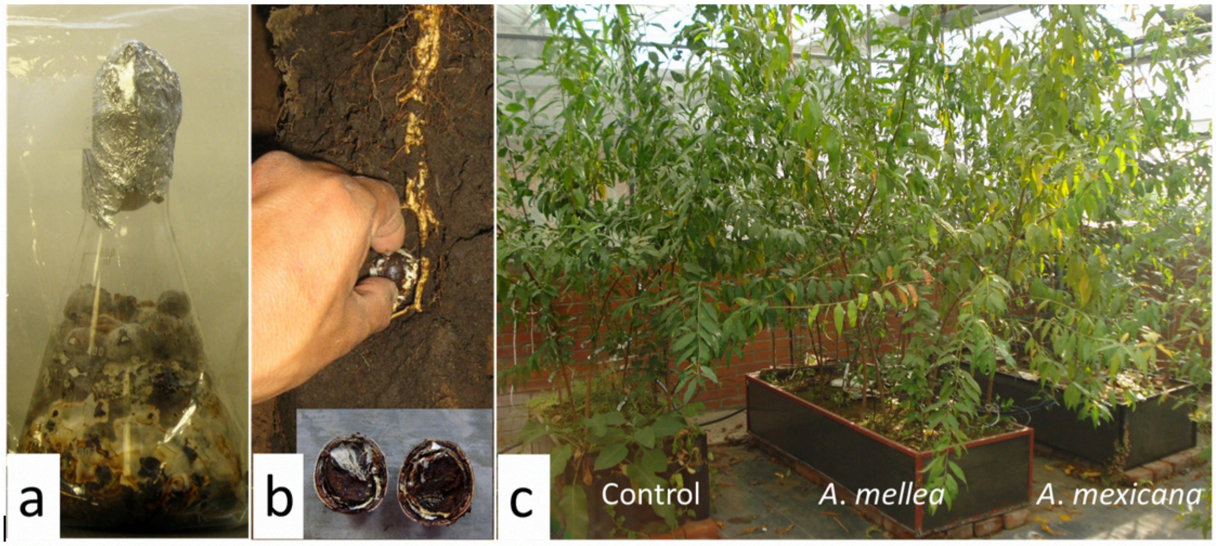

***Figure 1:*** *Rootstock inoculation with* Armillaria *spp. a) Seeds (acorns) of oak (*Quercus *sp.) used to increase* Armillaria *inoculum; b) Inoculation in roots; c)* Prunus *rootstock trial in greenhouse.*

**Results**

In the greenhouse at 22 months post-inoculation, 'Mondragon' showed a high resistance to *A. mexicana* and *A. mellea*, evidenced by the lowest averages with 2.5% IRI and 0% DRI. 'Criollos of La Goleta' and 'Nemaguard' showed high susceptibility to *A. mexicana*, and *A. mellea*, with similar values (21.6 and 24.6

IRI; 7.2 and 9.2 DRI, respectively) (Table 1). The *Armillaria* species used for inoculation produced no detectable differences for RDW, RV, RL, and PDW, but significant differences were noted among the rootstocks. The highest growth was observed in 'Criollos of La Goleta', followed by 'Nemaguard'. 'Mondragon' had the lowest values of RDW, RV, RL and PDW (data not shown). In all field evaluations (2013–2015), 'Mondragon' showed the highest resistance against *A*. *mexicana* (40, 59, and 66 months post-inoculation), which was displayed as the lowest values on WAS and average ranks (Table 2).

***Figure 2:*** Prunus *rootstocks test in field conditions. a) Inoculation of a tree with an acorn infected with* Armillaria*.*

***Table 1:*** *Percentage of infected and dead roots in three rootstocks inoculated with two* Armillaria *species and their combinations at 22 months post-inoculation in the greenhouse (From: Elías-Román et al. 2019).*

| **Rootstock/ *Armillaria* sp. Inoculated** | | **n¹** | **Infected root incidence (IRI) %²** | **Dead root incidence (DRI) %²** |
|---|---|---|---|---|
| 'Nemaguard'³ | | 18 | 24.6 a | 9.2 a |
| 'Criollos of La | | 18 | 21.6 a | 7.2 a |
| 'Mondragon'⁵ | | 18 | 2.5 b | 0.0 b |
| | | | | |
| *A. mexicana* | | 21 | 31.2 a | 13.1 a |
| *A. mellea* | | 21 | 17.5 b | 3.3 b |
| Control | | 12 | 0.0 c | 0.0 b |
| | | | | |
| *A. mexicana* | 'Nemaguard' | 7 | 49.2 a | 26.4 a |
| | 'Criollos of La Goleta' | 7 | 36.7 a | 12.8 b |
| | 'Mondragon' | 7 | 7.6 b | 0.0 c |
| *A. mellea* | 'Nemaguard' | 7 | 24.5 a | 1.2 bc |
| | 'Criollos of La Goleta' | 7 | 27.9 a | 8.7 bc |
| | 'Mondragon' | 7 | 0.0 b | 0.0 c |
| Control | 'Nemaguard' | 4 | 0.0 b | 0.0 c |
| | 'Criollos of La Goleta' | 4 | 0.0 b | 0.0 c |
| | 'Mondragon' | 4 | 0.0 b | 0.0 c |

¹Replications; ²Means followed by the same letters in a column are not significantly different, based on LS-means ($P <$ 0.05); ³*Prunus persica* × *P. davidiana* (Chinese wild peach); ⁴Landrace of *P. persica* (peach); ⁵*P. salicina* (Japanese plum)

***Table 2:** Armillaria disease severity on three peach rootstocks evaluated 6 years after planting in an orchard naturally infested with* Armillaria mexicana *in Coatepec Harinas, State of México, México. August 2009 to May 2014 (From: Elías-Román et al. 2019).*

| **Rootstocks** | **Disease severity**[1] | | | | | **Weighted average severity by rootstock** | **Average ranks**[2] |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** | | |
| **Disease severity 2013**[3] | | | | | | | |
| 'Japanese apricot'[4] | | 0 | 2 | 1 | 4 | 3.3 | 2.71 a |
| 'Criollos of La Goleta'[6] | 2 | 0 | 3 | 0 | 2 | 2.0 | 1.79 ab |
| 'Mondragon'[7] | 2 | 2 | 3 | 0 | 0 | 1.1 | 1.50 b |
| **Disease severity 2014**[8] | | | | | | | |
| 'Japanese apricot' | 0 | 0 | 1 | 0 | 6 | 3.7 | 2.57 a |
| 'Criollos of La Goleta' | 0 | 0 | 1 | 0 | 6 | 3.7 | 2.36 a |
| 'Mondragon' | 2 | 2 | 2 | 1 | 0 | 1.3 | 1.07 b |
| **Disease severity 2015**[9] | | | | | | | |
| 'Japanese apricot' | 0 | 0 | 0 | 0 | 7 | 4.0 | 2.5 a |
| 'Criollos of La Goleta' | 0 | 0 | 0 | 0 | 7 | 4.0 | 2.5 a |
| 'Mondragon' | 2 | 1 | 3 | 1 | 0 | 1.4 | 1.0 b |

[1]Individual disease severity visually assigned to five (0-4) modified classes of disease severity similar that reported by Metaliaj et al. (2006); [2]Average ranks with different letters are significantly different (alpha=0.0.5); [3]Disease severity observed at 40 months post-inoculation (Source: Elías-Román, 2013); [4]*P. mume* (Japanese apricot); [5]Number of trees in each class of disease severity; [6]Landrace of *P. persica* (peach); [7]*P. salicina* (Japanese plum); [8]Disease severity observed at 59 months post-inoculation; [9]Disease severity observed at 66 months post-inoculation.

## Discussion

Armillaria disease incidence/severity varied among the tested rootstocks, which could be attributed to differences in tolerance/resistance of *Prunus* host genotypes, differences in virulence of the *Armillaria* species/isolates used as inoculum, and/or other environmental interactions.

Under greenhouse conditions, *A. mexicana* was found to be more virulent than *A. mellea* across all rootstocks tested. *Armillaria mexicana* is a recently described species (Elías-Román et al. 2018); however, its distribution is already known to include important peach-producing regions, such as State of Mexico (Elías-Román et al. 2013) and Michoacan (Rivas-Valencia et al. 2017).

The full geographic and host range of *A. mexicana* is largely unknown and warrants further study. This study further confirms the role of *A. mexicana* as a primary root pathogen that causes extensive damage/mortality in peach trees in central Mexico, which is perhaps attributable to its higher virulence with respect to *A. mellea*, and its greater distribution (Elías-Román et al. 2013).

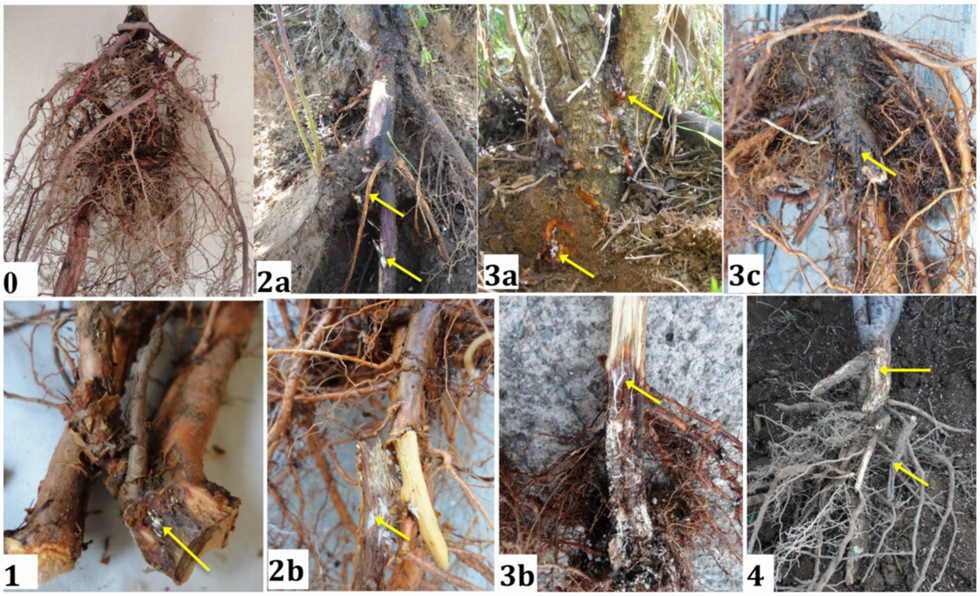

***Figure 3:*** *Armillaria disease severity scale of five classes (0 to 4) in* Prunus *spp. exposed to* Armillaria *in the field, where: 0 = Healthy tree. 1 = Tree without disease symptoms or dead, but with one or more primary or lateral roots with initial colonization by a mycelial fan of* Armillaria*; 2 (a-b) = Tree without disease symptoms in the foliage, presence or absence of gummosis in root crown and/or stem, and living primary or secondary root, colonized with subcortical mycelial fan of* Armillaria*, and presence or absence of* Armillaria *rhizomorphs (2a), dead primary or secondary root, colonized with subcortical mycelial fan of* Armillaria*, and presence or absence of rhizomorphs (2b); 3 (a-c) = Tree with or without above-ground disease symptoms (gummosis, chlorotic leaves), subcortical colonization of* Armillaria *mycelial fans in the root crown and/or one or more roots (3a and 3b), one or more dead roots infected by* Armillaria*, presence or absence of rhizomorphs (3c); and 4 = Dead tree. Arrows show symptoms and signs of* Armillaria*. (From: Elías-Román et al. 2019).*

## Conclusions

Work to develop *Armillaria*-resistant rootstock, such as ‘Mondragon’, is an integral part of management strategies for Armillaria root disease of peach in Mexico. Integrated disease management strategies, such as excavating root collars above ground level (Schnabel et al. 2012) and inoculum reduction measures (Cox et al. 2005b), can be used in conjunction with *Armillaria*-resistant rootstock to improve overall production.

For each production area, it is critical to determine which *Armillaria* spp. are present. The *Armillaria* genus displays considerable variation in virulence, and different *Armillaria* species represent different threats for different hosts and geographic areas. In peach orchards in central Mexico, *A*. *mexicana* appears to be the primary pathogen, which must be considered for disease management strategies. Furthermore, management strategies for Armillaria root disease should also consider other factors, such as climate, soil types, technology, economics, and other environmental factors.

## Acknowledgments

The National Council for Science and Technology (CONACYT-México) supported the initial part of this study. We thank Dr. Dionicio Alvarado Rosales, Dra. Alejandra Almaraz Sánchez, Mr. Rigoberto Mondragón, Ing. Rodolfo B. Muñoz Perez, Dra. Esmeralda Judith Cruz Gutiérrez, and MSc. Oscar Alejandro Martínez-Jaime for their important contributions to this study.

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# IDENTIFICATION OF GENETIC GROUPS WITHIN THE INVASIVE BROWN ROOT ROT PATHOGEN, *PYRRHODERMA NOXIUM* (FORMERLY *PHELLINUS NOXIUS*)

**Andrea R. Garfinkel[1], Phil G. Cannon[2], Ned B. Klopfenstein[3], Jane E. Stewart[1], and Mee-Sook Kim[4]**

## Abstract

Recent taxonomic investigation into genus *Pyrrhoderma* has determined that the genus currently contains six species, including *Pyrrhoderma noxium* (formerly *Phellinus noxius*), and one unnamed, but likely novel species (Zhou et al. 2018). *Pyrrhoderma noxium* is considered one of the most important pathogens in this genus due to its worldwide distribution in pan-tropical regions, including Asia, Australia, Africa, and Oceania. *Pyrrhoderma noxium* has a wide host range of over 200 plant species and causes mortality of >100 tropical tree species (e.g., breadfruit, mango) that are integral to economies, native cultures, and forest ecosystems of the Pacific Islands (e.g., Ann et al. 2002). In a previous study, over 100 isolates of *P. noxium* (as *P. noxius*) were collected from the Pacific Islands (e.g., Guam, Rota, Saipan, Pohnpei, Yap, Palau, Kosrae, and American Samoa) and other locations (e.g., Japan, Taiwan, Hong Kong, Malaysia, and Australia) and phylogenetic analyses were conducted based on ITS (internal transcribed spacer) and LSU (28S nuclear large sub unit) rDNA gene sequences. Results from this study found that both the ITS and LSU regions provide strong evidence that *P. noxium* isolates differ from the ex-type culture of *P. noxium*, suggesting that *P. noxium* isolates potentially represent one or more undescribed species or distinct genetic groups. Our current work aims to identify the appropriate taxonomic status for the previously characterized distinct genetic groups of *P. noxium*. Accurate identification of species and/or genetic groups is essential to understanding the true host range and geographic distribution of brown root rot pathogens that are currently comprised within *P. noxium*. Further, correct identification of the pathogen species or sub-species is critical to develop subsequent measures for detecting and managing the pathogen and its spread, such as creating and enforcing quarantine measures, especially for the widespread and devastating pathogens that are currently considered as *P. noxium*.

## Introduction

- The genus *Pyrrhoderma* has recently undergone taxonomic restructuring, including the addition of two species formerly within genus *Phellinus*, and now is made-up of six named and one unnamed species (Zhou et al. 2018).
- *Pyrrhoderma noxium* (formerly *Phellinus noxius*) is an aggressive pathogen (Figure 1) on over 100 tropical tree species, including many of which are ecologically, culturally, and economically valuable (e.g., Ann et al. 2002, Brooks 2002, Sahashi et al. 2015).
- Over 100 isolates of a *Pyrrhoderma* species were collected across the Pacific Islands (including Guam, Rota, Saipan, Pohnpei, Yap, Palau, Kosrae, and American Samoa) and other locations where this pathogen is present (such as Japan, Taiwan, Hong Kong, Malaysia, and Australia).
- The taxonomic placement of these isolates is unknown in the context of the newly-revised phylogenies.

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***Figure 1:*** *Dried mycelial mat of* Pyrrhoderma noxium *on a moso'oi (*Cananga odorata*) tree in American Samoa (Photo: Fred Brooks).*

## Goal

To correctly identify and characterize the collected isolates of *Pyrrhoderma* by phylogenetic analyses that include the sequences of type specimens and other related species described in the literature.

## Materials and Methods

- DNA extraction of 95 isolates of *Pyrrhoderma* species from around the world.
- PCR and sequencing of the ITS (internal transcribed spacer), LSU (28S nuclear large subunit), *RPB2* (RNA polymerase II), and *tef1* (translation elongation factor – 1 alpha) genes of selected isolates.
- Alignment and trimming of sequences using Clustal W implemented in MEGA 6.
- Maximum likelihood (implemented in MEGA 6) and Bayesian analysis (implemented in Mr. Bayes).

## Results

- Analysis of the ITS and LSU loci suggests the *Pyrrhoderma* isolates (Figure 2; white circles) are not members of *P. noxium* (Figure 2; blue circles), as previously described (Figure 2) and additional isolates in GenBank characterized as *P. noxium* do not align with the type specimen, but with our isolates (data not shown).
- Our collection of *Pyrrhoderma* isolates (Figure 2; white circles) are more closely related to the unnamed *Pyrrhoderma* species (Figure 2; green circles) partially described by Zhou et al. (2018).
- *RPB2* and *tef1* (data not shown) suggest notable genetic diversity among our isolates and up to three phylogenetic species and/or groups may be present (Figure 3). A group from American Samoa (indicated in green branches) appears to be genetically distinct from other geographic populations (Figure 3).

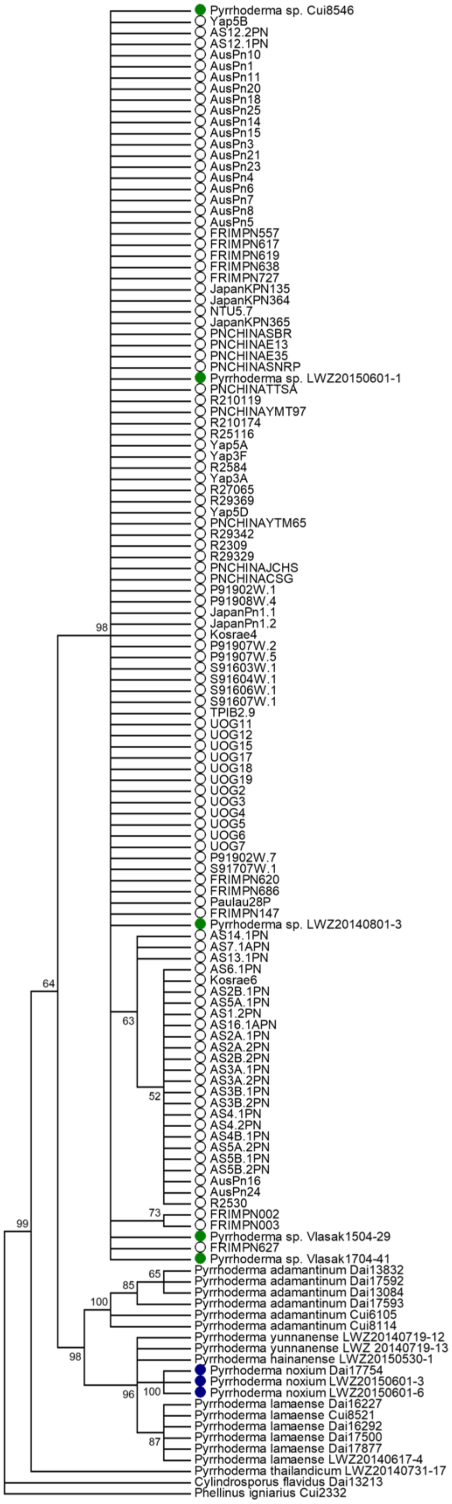

***Figure 2:*** *Combined ITS+LSU ML tree describing the relationship of* Pyrrhoderma *sp. isolates collected globally (white circles) to voucher specimens (blue circles) described by Zhou et al. (2018). The unnamed* Pyrrhoderma *species (green circles) was partially described by Zhou et al. (2018).* Cylindrosporus flavidus *and* Phellinus igniarius *are used as outgroups. The Hasegawa-Kishino-Yano model with gamma distribution was used to model evolutionary rates. Branches with less than 50% bootstrap support are not shown. Bootstrap replicates = 1000.*

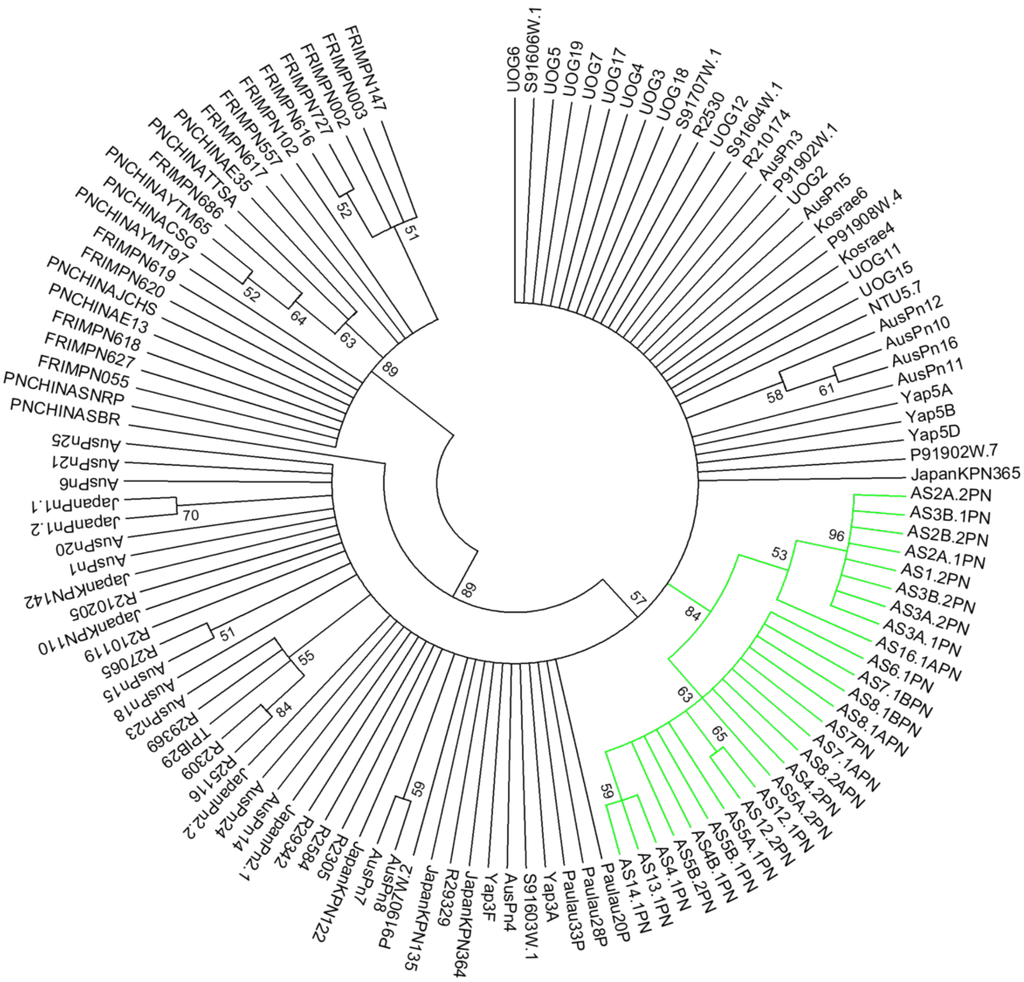

***Figure 3:*** *RPB2 maximum likelihood tree describing genetic diversity among* Pyrrhoderma *sp. isolates previously collected around the world. A group from American Samoa (indicated in green branches) appears to be genetically distinct from other geographic populations. The Kimura 2-parameter model was used to model evolutionary rates. Branches with less than 50% bootstrap support are not shown. Bootstrap replicates = 1000.*

## Implications

- Multiple *Pyrrhoderma* species besides *P. noxium* may be important pathogens of tropical and subtropical forests.
- These multiple species may differ in host range, virulence, ecological niches, environmental requirements, or other factors that may influence the epidemiology of the diseases they cause and their management.
- The putatively novel *Pyrrhoderma* species suggested by this study has not been reported in some U.S. states/territories (e.g., Hawaii, Florida, Puerto Rico), where it represents a major invasive threat based on bioclimatic models (Stewart et al., in revision).
- Taxonomy and correct species identification are key to understanding hosts and geographical range, which are important for establishing regulatory processes, such as quarantines, that can help prevent pathogen spread.

## Future Goals

- Additional genetic analyses to confirm any new species present in our collection.
- Morphological characterization of the isolates to corroborate genetic data.
- Development of molecular detection methods to identify the pathogen (or genetic groups) and evaluate management treatments in tropical forests.

## Acknowledgements

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# RADIO FREQUENCY BACKGROUND AND MICROBIOME EFFECTS ON HEALTH OF ASPEN (*POPULUS TREMULOIDES*): COULD THESE FACTORS UNDERLIE FOREST DECLINE?

**Katie Haggerty[1], Jani Little[2], and Sam Miller[3]**

## Abstract

This study was designed to investigate the effects of the background of anthropogenic radio frequency (RF) pollution on the health and growth of aspen and on aspen/microbiome symbiosis. Forest decline was first seen and has been increasing since the mid-19th century. Sudden aspen decline was identified in 2004 in Colorado. From 1800 up to the present, generation and use of electricity has also increased and has radically changed the earth's electromagnetic (EM) environment. Plants have been shown to respond to various frequencies of low level, non-thermal RF exposure as they do to physical injury or burning. The experiment compared health and growth outcomes for aspens exposed to the ambient RF background with the same parameters in RF shielded aspens. Inoculation with symbiotic fungi was the second independent variable. The results indicate that RF pollution can impair aspen/microbiome symbiosis, inhibit photosynthesis, reduce fall nutrient storage, and shift timing of dormancy, leaving aspens more vulnerable to environmental challenges including conditions of temperature extremes. Exposure to ambient RF background in this experiment is statistically associated with summer leaf necrosis and leaf loss consistent with SAD.

## Introduction

In this paper we build on recent studies that have shown that low level, non-thermal exposure to various radio frequency (RF) fields can induce bio-chemical stress reactions in plants like those caused by injury or burning (Roux et al. 2006) and affect metabolic and reproduction rates in in fungi (Berg & Berg 2006). Plant and fungal integral membrane ATPases and voltage gated ion channels may be directly affected by interference from external anthropogenic RF fields (Blank 2005, Romanenko et al. 2017, Taghian et al. 2015). Because RF background pollution is now present world-wide, it could be a factor in forest decline.

Symbiosis with mycorrhizal fungi is essential for the health and growth of most plants (Behie & Bidochka 2014). In tree species, fungal symbiotes provide mineral nutrition, facilitate water acquisition, and provide some protection from pathogens (Parniske 2008, Azcon-Aguilar 1997). Loss or impairment of the symbiotic microbiome over time on large geographic scales could cause symptoms of forest decline that have been observed globally. Symptoms of forest decline can include: slow growth; sparse, small, often chlorotic foliage; premature autumn color; premature leaf drop; distress crops; reduced storage of nutrient reserves; dieback of twigs and branches; and adventitious sprouts following branch dieback (Sinclair 2005).

The phenomenon of forest decline (Manion & Lachance 1992) has been the object of extensive study, and many factors, and interactions of factors have been investigated as causal agents. Well documented instances of forest decline have occurred since the mid-19th century worldwide (Kandler 1992, Hennon et al. 1990). Onset of decline in central Europe occurred rapidly beginning in the late 1970's. Bio-indicators of decline have been catalogued (Sinclair 2005, Schütt 1989) and possible agents of decline proposed (Klap et

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al. 2000, McGlaughlin 1987). Study of forest decline continues to add to the documentation of the extent of decline events, understanding of interaction of biotic and abiotic environmental factors, and the investigation of possible underlying causes (Hogg et al. 2005, Filip & Rosso 1999, Phillips & Gentry 1994). In early studies, a new agent was not identified to account for extensive decline observed in Europe and the eastern US, but forests seemed to have become more vulnerable to previously existent biotic and abiotic agents, including conditions of temperature and freezing (Houston 1992, Braathe 1957, Pomerleau 1991). Water stress, attributed to climate warming and drought, is often suggested as a factor in forest decline. More frequent winter freeze-thaw events, also attributed to climate warming and which may cause damage to the water transport capacity of trees (Houston 1992), have been cited as a factor in drought intolerance and decline. In his study of Alaska yellow cedar decline, Hennon et al. (1990) note that freeze damage occurred at lower elevations, and trees in higher locations were not affected. McLaughlin et al. (1987) observed that extensive red spruce freeze damage in the 1960's occurred in much less severe winters than the previous cold weather of 1880, which preceded an incident of decline in red spruce. Other authors note similar reduced freeze tolerance (Schaberg 2008). These reports suggest that a previously unrecognized factor may underlie increased susceptibility to freezing. A large study of mycorrhizal activity in European forests found that beneficial microbial activity in the soil has diminished in recent decades (van der Linde et al. 2018). Separate studies of European forest decline found widespread mineral deficiencies in trees (Veresoglou et al. 2014, Jonard et al. 2015) consistent with diminished symbiotic fungal activity (van der Linde et al. 2018) that could underlie decline. A recent study found that, in all dominant tree species in old growth forests in the western US, tree mortality has been doubling every 18- 29 years, and mortality rates are now higher than recruitment (van Mantgem et al. 2009). Similar trends and changes in forest demographics are seen in forests globally (Lewis et al. 2004, Ciesla & Donaubauer 1994).

Very rapid decline of a number of tree species worldwide has been occurring since about 2000 (Breshears et al. 2005, Rizzo & Garbelotto 2003, Stokstad 2019). Again, water deficit, or lack of water conductance, appears to be a common factor identified in these incidents. Aspen decline was observed in North America beginning in the mid-20th century (Basham 1958). Sudden aspen decline (SAD) was first documented in Colorado in 2004. In Worrall's 2008 study, SAD in Colorado was found to be associated with stand maturity, low stand density, and south-facing lower elevation sites. Although various common pathogens were present, leaf loss was not caused by those agents. No other site characteristics or biotic factors in that study were found that could account for the sudden synchronous nature of the decline. Resistance to SAD was statistically associated with slenderness in the ratio of aspen stem height to diameter at breast height (Worrall et al. 2008).

### RF and microbiome hypothesis

Our experiment is one in a series of similar experiments which began in 2005. The anthropogenic RF environment has been increasing from that time up to the present, so that RF exposure in these experiments has increased each year. In the first years (2005-2007), almost all types of plants shielded from ambient RF responded with vigorous growth. In our 2007 experiment with aspen, RF shielded aspens showed more growth and brighter autumn coloration compared with RF exposed seedlings (Haggerty 2010). That year, necrosis appeared mainly in RF exposed fall senescing leaves. Our assumption at that time was that the RF effect was on the plants directly. In retrospect, it seems that plants from 2005-2007 had a symbiotic microbiome, but it was suppressed by exposure to ambient RF fields. So, by shielding from RF background in those years, function of the fungal symbiotes and growth of the aspens were released. In contrast, from 2009-2015, aspens and other plants did not respond to RF shielding. In those years, growth and health of RF shielded and exposed plants did not differ. Both treatment types, RF shielded and RF exposed, showed stunted growth, pale coloration, leaf tissue necrosis, and lack of bright fall color. In those years it seems, the microbiome was not generally available in the environment or on the plants used in the experiment. In the spring of 2016, both RF shielded and RF exposed maple (*Acer rubrum*) seedlings were treated with a symbiotic fungal inoculant, and the RF shielded seedlings responded with vigorous growth. The RF exposed maple seedlings remained quite small relative to the RF shielded plants. Again, in the 2018 experiment with aspen, application of the fungal inoculant MycoGrow was beneficial to aspen only when the plants were RF shielded. When setting up our experiment in April 2018, small aspen seedlings were not available for our experiment. The Colorado Forest Service nursery in Fort Collins did have stunted dormant aspen plugs in Styro-blocks that averaged about 24 cm in height. They looked like fist year seedlings but were "at least six years old" according to the nursery manager. These plants were from the last crop started at the nursery. They were kept in the shade house, had been watered, had live roots, and produced small leaves but had not increased in height since their first year. Prior to 2010, the COFS nursery raised a crop of aspen seedlings each year. After that time, aspen seedlings did not grow well at the nursery and their production was outsourced to the USFS greenhouse in Gunnison. The stunted aspens seemed like a good test case for our hypothesis, though we were concerned that they might not survive. Early in April 2018, 36 stunted aspen plugs, still in the styro-blocks, were placed in a Faraday cage at the experiment site, and all were inoculated with MycoGrow. They began to leaf out April 17 and continued to grow vigorously through May. They averaged 24 cm in height April when purchased, and 55 cm by August 2019. So, RF shielding combined with Myco-inoculation released the growth of these stunted aspens.

The electromagnetic (EM) environment of the earth has changed radically in the past two centuries due to human experimentation with and use of electricity for industry, communications, transportation, and lighting. Radio frequencies are EM frequencies below 300 GHz with wavelengths longer than those of visible light and infrared. Radio frequency (RF) energy can disperse over great geographic areas, and because RF sources are numerous and widespread, there is a background of RF noise covering the earth. Viewed from satellite in various frequencies, this background pollution is seen to cover continents fairly evenly, like a cloud, though its intensity is greatest in areas with higher human population (Burr et al. 2004, Figure 1). By comparison, visible light sources seen from space at night are readily identified as human population centers and highways. RF background, then, is present globally, even in "pristine" wilderness areas. This background radiation is presently ten to one hundred times stronger than the earth's naturally occurring EM environment below 30MHz, and signal intensities across the RF spectrum are in the range of $10^4$ to $10^7$ times the earth's naturally-occurring RF environment (Sanders 1998). The anthropogenic RF background (which contains low levels of frequencies across the RF spectrum) has generally been assumed to be harmless to living things and has been usually disregarded in earlier experiments designed to investigate possible RF effects.

Plants, fungi, and all living things can be seen as electrical/electrochemical systems: internal functions and signal transmission within organisms are powered by voltage/ion concentration differentials across cell membranes. This potential energy is maintained by integral plasma membrane enzymes, ATPases (Falhof et al. 2016). The release of ion concentration through voltage-gated ion channels in plants powers growth, internal signal transmission, plant movement, opening of stomata, and transport of nutrients: functions that maintain health and trigger response to challenges from the external environment (Duby & Boutry 2009, Zeiger 1983). These same electrical systems are present in fungi and facilitate communication and nutrient transfer between fungal symbionts and their host plants (Krajinski et al. 2014, Wang et al. 2014, Hubberten et al. 2015).

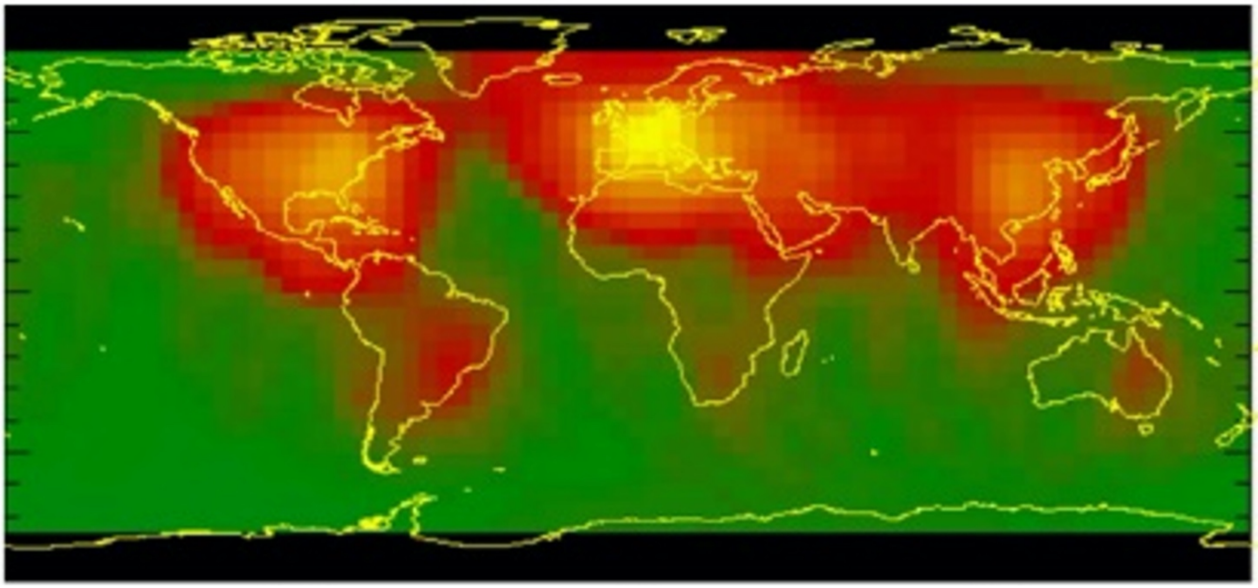

***Figure 1:*** *Forte Satellite: 131 MHz RF background 2000. Areas of highest radio background intensity occur in the eastern United States, central Europe, and China. Acquired by the Los Alamos National Laboratory FORTE spacecraft. Principal investigator A.R. Jacobson.*

A number of recent studies with plant models have demonstrated that exposure to non-thermal human generated RF fields of various frequencies that are common in the present technological environment can cause biochemical-stress responses in plants like those elicited by injury, burning, and virus or bacterial attack (Roux et al. 2006, Vian et al. 1996). Temporary cessation of water uptake can occur in plants exposed to physical injury, weak flame, rapid change in ambient light levels (Davies et al. 1991), viral or bacterial pathogens (Liu et al. 2009), or electrical stimulus (Bose 1923). Stress related mRNA is generated at the site of an injury or RF exposure and rapidly communicated to the entire plant by ion fluxes, triggering stress response throughout the plant (Beaubois et al. 2007). This stress response induces reduction in ATP levels (Roux et al. 2006), causing reduced plant metabolism (Vian 1996). Other effects of various RF exposures demonstrated in plants include increased growth (Reed et al. 1993), reduction in photosynthesis (Hirano 1998, Sandu et al. 2005), reduced growth (Kumar et al. 2016), reduced seed germination (Tkalec et al. 2009, Halgamuge et al. 2015), changes in morphology (Tafforeau et al. 2004, Bitonti et al. 2006), changes in enzyme activity (Kouzmanova et al. 2009, Sharma et al. 2010), inhibition of early seedling growth, and perturbation of carbohydrate metabolism (Kumar et al. 2016). In fungi, RF exposure can increase or decrease reproduction and metabolic rates (Berg & Berg 2006)

## Methods

The experiment was conducted on Boulder County Parks and Open Space land, in a rural area bordering Roosevelt National Forest near Lyons, CO at an altitude of 1700 m. The site was located at 40.2°N latitude and -105.30°W longitude on a south facing upper slope overlooking the South Saint Vrain River. The ecological system of the area was Rocky Mountain lower montane. Vegetation included wild grasses and low shrubs: rabbit brush (*Ericameria nauseosa*), sage (*Artemisia tridentate*), skunk brush (*Rhus trilobata*), with nearby scattered ponderosa pine (*Pinus ponderosa*). There were no near sources of anthropogenic RF at the site (Figure 2).

***Figure 2:*** *BCPOS 2018 Experiment site looking south.*

Four treatment types with three repetitions of each were used in the experiment: Six Faraday cages were used to block the ambient RF background. Each of these 70x70x110 cm enclosures was composed of two layers of aluminum window screen supported by a light bamboo frame. This configuration, two layers of aluminum screen, was tested at a NIST laboratory and found to attenuate signals from 1.0 MHz to 3.0 GHz by about 40 dB, a factor of 10,000. A 9x6 m plot was divided into a grid of 6 cells. Treatment enclosures, RF exposed and RF shielded, were randomly assigned, one per cell, and centered within their cells. Each Faraday cage rested on a plywood base placed flat on the ground. In three of these cages, ambient RF noise was introduced to the interior passively by connecting an antenna outside the cage (receiving) to an antenna inside (transmitting) with coaxial cable. Aspen plants were the objects of the experiment and received the treatments. Because in our previous experiment with aspen, differences in growth between RF shielded and exposed groups were striking (74% and 60% for stem length and leaf area, respectively) and consistent within treatment groups (Haggerty 2010), a sample size of 36 aspens seemed adequate. In late May, 36 aspen plugs, averaging 24 cm in height, were potted in gallon pots in sterile commercial potting soil. These plants were purchased from the Colorado Forest Service nursery in Fort Collins, CO. Aspens were raised from seed and each plant was genetically distinct (see Discussion). The aspens were treated with MycoGrow in early April to promote their growth. Before the experiment began (May 27 and June 2, 2018) all aspens were exposed to ambient RF for a total of four hours, in order to eliminate the Myco-inoculant. The aspens were then potted and assigned to treatments. The plants were randomly assigned to treatment types, six pots per cage. Aspens within each cage were then divided into two groups of three plants – a group of three at each end of each treatment cage. In each cage, one group of three was inoculated with symbiotic fungal spores and one was not. The group to be inoculated was chosen randomly. MycoGrow, from Fungi-Perfecti, was used as the inoculant. Plants were watered liberally, as needed. No fertilizer was used during the experiment. Initial data on length of new spring leader growth and number of leaves were taken June 2, 2018 when the experiment started. On August 11, 2018, data were taken on leader lengths and leaf widths for each aspen plant, as well as presence or absence of leaf tissue necrosis. Plant leaf color during the experiment was observed and noted. Response to freezing was recorded in October 2018. From April 20 through May 23, 2019 data were recorded on number of open leaf buds per plant per day.

This study had a sample size of 36 aspen plants, and two-way analysis of variance was used to test for treatment effects. The two independent variables were 1) Two levels of exposure to ambient RF and 2) the presence or absence of the symbiotic fungal inoculant. There were four outcome measures taken on each aspen plant (stem length, leaf widths, number of leaves, and presence or absence of leaf tissue necrosis). So, there was a total of four dependent variables. Analysis of variance was done on the four dependent variables separately as well as a single model that simultaneously analyzed all dependent variables using the SPSS Multivariate Analysis of Variance (MANOVA) module. A posteriori tests were conducted to determine which of the treatments showed significantly different effects on plant health and behavior. Based on our previous studies, the data was expected to confirm the hypothesis that aspens that were both treated with the fungal inoculant and RF shielded would grow more vigorously, be resistant to leaf necrosis, have stronger fall coloration and greater resistance to freezing, and that their timing of fall dormancy and spring bud burst would occur slightly later than the RF exposed groups and RF shielded plants that were not Myco-inoculated.

## Results

Date of the onset of leaf necrosis was July 5, 2018 (Figure 3). Necrosis increased and was followed by significant loss of leaves due to necrosis in July (Figure 4). This progression of symptoms, consistent with SAD, occurred in treatment groups that were RF exposed whether or not they were treated with MycoGrow and in one RF shielded group that was not treated with the inoculant. Chlorosis of leaf tissue in RF exposed groups was observed beginning June 21, preceding onset of necrosis. After leaf loss due to necrosis, several RF exposed stems produced small, multiple adventitious leaf sprouts. No summer leaf chlorosis or necrosis occurred in aspens that were both RF-shielded and inoculated with MycoGrow. These shielded plants had significantly less necrosis ($p = 0.035$, Figure 5) and lost significantly fewer leaves during the growing season than RF exposed groups ($p = 0.03$, Figure 6). Lack of fall coloration, susceptibility to freezing, and loss of leaves in late September – early October 2018 were observed in RF exposed groups. Radio frequency shielded aspens developed strong fall coloration and were resistant to freezing, and abscission of leaves occurred mainly in mid-October 2018 (Figure 7). The timing of spring bud burst in 2019 was advanced in RF exposed and non-MycoGrow groups relative to the RF shielded-MycoGrow groups (Figure 8). Growth differences between treatment groups (leader length, number of leaves, and leaf widths) were not statistically significant.

***Figure 3:*** *July 5, 2018, leaf tissue necrosis began to appear in RF exposed groups.*

***Figure 4:*** *Significant loss of leaves due to necrosis occurred in July 2018 in RF exposed groups and in one RF shielded non-Myco-inoculated group.*

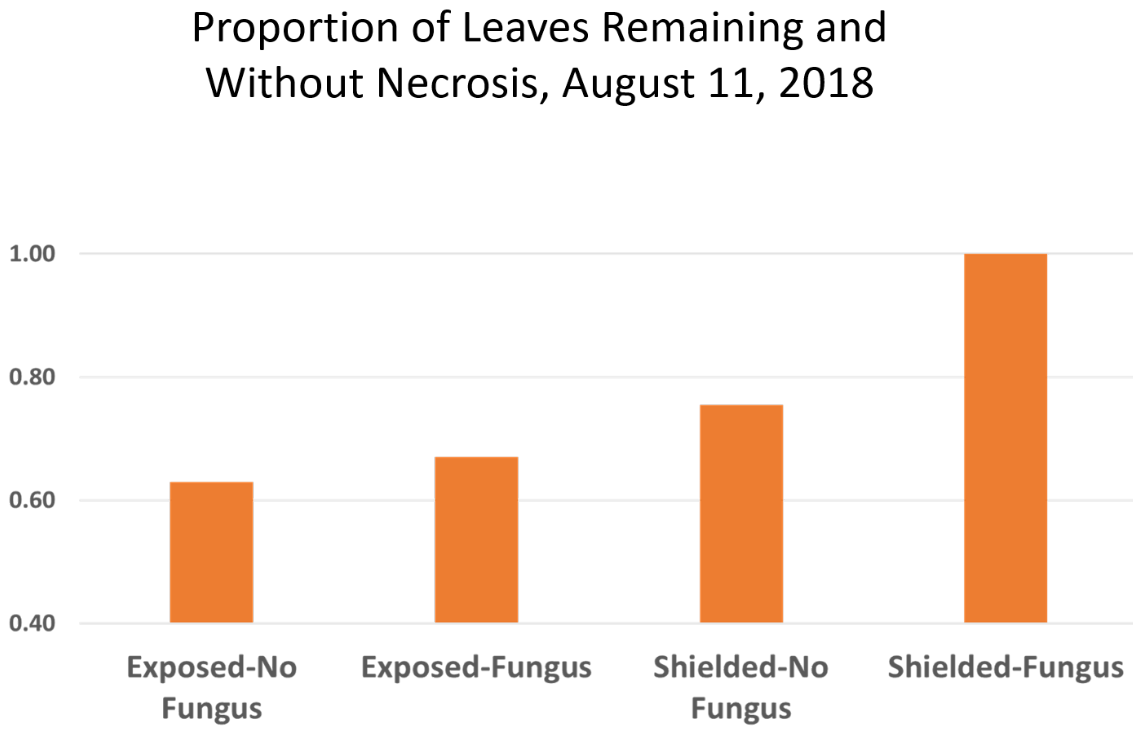

***Figure 5:*** *The number of leaves affected by necrosis, including leaves dropped due to necrosis, was statistically significant in RF exposed groups relative to RF shielded-Myco inoculated groups (p = 0.035).*

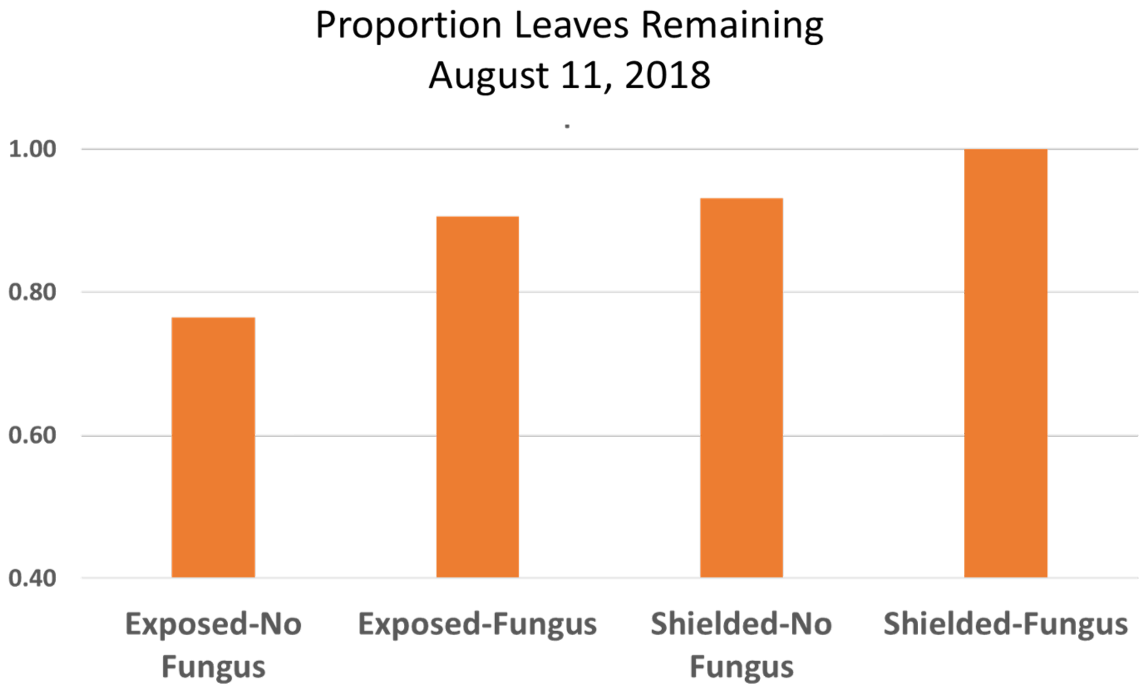

***Figure 6:*** *Leaf loss in RF-exposed groups was statistically significant relative to RF-shielded Myco-inoculated groups (p = 0.03).*

## Discussion

Leaf necrosis and leaf loss consistent with SAD occurred in this experiment. Because conditions were controlled and closely monitored, it was possible to look at various factors and determine the probability of their contribution to the observed decline: Watering was checked daily and plants were watered liberally throughout the experiment. All treatments had the same watering regime, and water deficit did not occur. The treatment enclosures, made of two layers of aluminum screen, attenuated ambient light by about 30%. Because the six Faraday cages were identical, light intensity and quality, air-flow, and heat retention were also equal among the treatments, so those factors did not contribute to differences in outcomes among treatment groups. Freeze damage is often suggested as a factor in forest decline which may negatively affect water uptake and conductance. Aspens used in the experiment were all from the same group of seedlings from the COFS nursery in Fort Collins. When the experiment was set up, these plants were randomly assigned to treatments. If freeze damage had been present in the seedlings, its effects would have appeared in all treatments without statistical difference.

Differences between treatments occurred in timing and quality of dormancy: Advanced timing of spring bud burst seen in RF exposed and RF shielded-non-Myco-inoculated compared to RF shielded-Myco-inoculated aspens, would make the former groups more vulnerable to spring freeze damage. RF shielded-Myco-inoculated seedlings had later, more synchronous bud burst timing which appeared to be less sensitive to ambient temperature (Figure 8). Lack of fall dormancy response, i.e.: lack of strong fall coloration (anthocyanin pigmentation), early leaf loss, and lack of freeze tolerance in RF exposed compared to RF-shielded-Myco-inoculated groups, suggests impaired fall nutrient transport and storage, which could negatively affect plant fitness in the spring. A study comparing mutant non-anthocyanin-producing strains of fall anthocyanin-producing species *Cornus sericea, Vaccinium elliottii,* and *Viburnum sargentii* showed very similar results to this experiment with regard to fall color in that extensive necrotic leaf tissue was seen in non-anthocyanin-producing mutants during fall leaf senescence. Fall transport and storage of nutrients was also impaired. Wild-type strains of those species which produced anthocyanins did not exhibit necrotic lesions (Hoch et al. 2003).

***Figure 7:*** *Lack of fall coloration, susceptibility to freezing and loss of leaves in September – early October 2018 were observed in RF exposed groups (left image); Shielded-Myco-inoculated aspen developed strong fall color and were resistant to freezing. Most leaves dropped during the third week in October in these groups (right image).*

Chlorosis preceded the onset of SAD in in this experiment. Chlorosis is a possible indicator that inadequate mineral nutrition is present to synthesize chlorophyll. Reduced photosynthesis would reduce production of sugar. This disruption in carbohydrate metabolism could make aspen less viable partners in symbiosis with fungi, because plants exchange carbohydrates for mineral nutrients and water (Kiers et al. 2011, Hodge & Fitter 2010). Direct RF effects on fungi changing their metabolic and reproduction rates could also be a factor negatively affecting symbiosis and therefore plant nutrition. Only RF shielded aspens benefited from application of the symbiotic fungal inoculant, were able to maintain symbiosis, and were not subject to chlorosis.

Symptoms of SAD, leaf tissue necrosis and leaf loss, resemble lack of water or lack of adequate water conductance. Electrical stimulus or physical injury in plants, as mentioned earlier, can temporarily stop or slow water uptake and transpiration (Davies et al. 1991, Bose 1923). In hot weather, temporary lack of transpiration could cause leaf tissue necrosis due to inability to moderate tissue heating by evaporation. The combination of high ambient temperatures, increased intensity of RF exposure causing stress response and reduced transpiration, and loss of symbiotic fungal microbiome and its water regulation capacity could account for leaf necrosis and summer loss of foliage observed in this experiment. Early vigorous spring growth promoted by spring application of MycoGrow (subsequently removed by RF exposure) may explain why growth differences from June 2 to August 11, 2018 in this experiment were not statistically significant.

Human-generated RF pollution began to be present in about 1800, and has been increasing since that time. Anthropogenic RF background is now present across the planet, and has, in this experiment, been shown to cause negative effects on the health of aspens and their symbiotic microbiome. Thus, this previously uninvestigated underlying factor may explain, at least in part, global forest decline and changes in forest demographics that have been observed over the past two centuries.

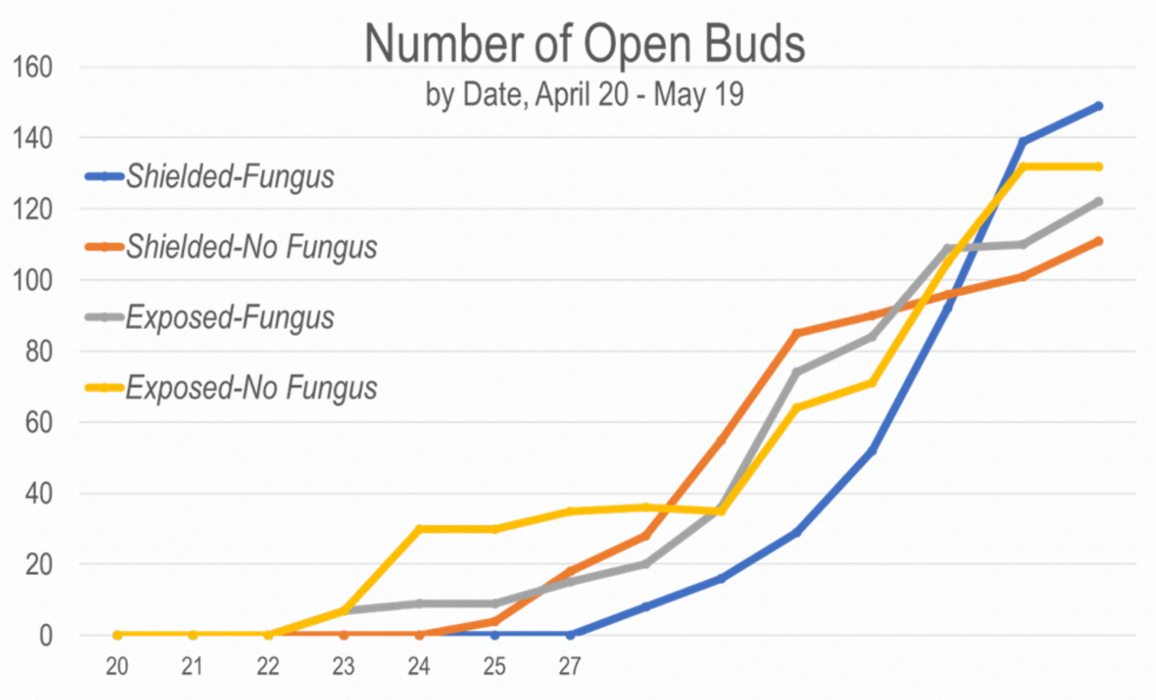

***Figure 8:*** *Spring bud burst in 2019 was advanced in RF exposed and non-Myco groups relative to RF shielded and Myco-inoculated groups.*

## Conclusion

The present RF background in rural Colorado appears to impair or destroy the symbiotic microbiome of aspen, and may affect the timing of dormancy. RF induced loss of fugal microbiome and direct effects of RF pollution on aspen appear to make them more vulnerable to conditions of extreme temperature. Results of this study indicate that RF exposure is a plausible underlying cause of SAD. Further investigation of this issue, in various climates and with a variety of plant models, is called for.

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# MAXIMUM ENTROPY-BASED BIOCLIMATIC MODELS PREDICT AREAS OF CURRENT AND FUTURE SUITABLE HABITAT FOR *ARMILLARIA* SPECIES IN WESTERN OREGON AND WESTERN WASHINGTON

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## Introduction

Climate change is predicted to increase the impacts of Armillaria root disease as host trees become maladapted to their environment, escalating tree stress, and potentially increasing susceptibly to *Armillaria* pathogens (e.g., Klopfenstein et al. 2009, Kliejunas et al. 2009, Kliejunas 2011, Sturrock et al. 2011, Dempster 2017, Kubiak et al. 2017, Aslam & Magel 2018). Powerful statistical bioclimatic modeling methods [e.g., Maximum entropy (Maxent)] are available to determine probable suitable climate space and potential distribution for *Armillaria* species for contemporary and future time periods, even with minimal presence data (Phillips et al. 2006, Pearson et al. 2007, Klopfenstein 2009a). Currently, only limited information is available on *Armillaria* spp. in western Washington and western Oregon, yet this region is expected to experience strong climate-change influences. In 2016, a collaborative project was initiated to determine the distribution of *Armillaria* species in the region under changing climates.

## Objectives

(1) Isolate, culture, and identify *Armillaria* species from collections/surveys throughout western Washington and western Oregon.

(2) Predict current suitable climate space (potential distribution) of six *Armillaria* species identified in the region and project the distributions for future time periods under several climate-change scenarios.

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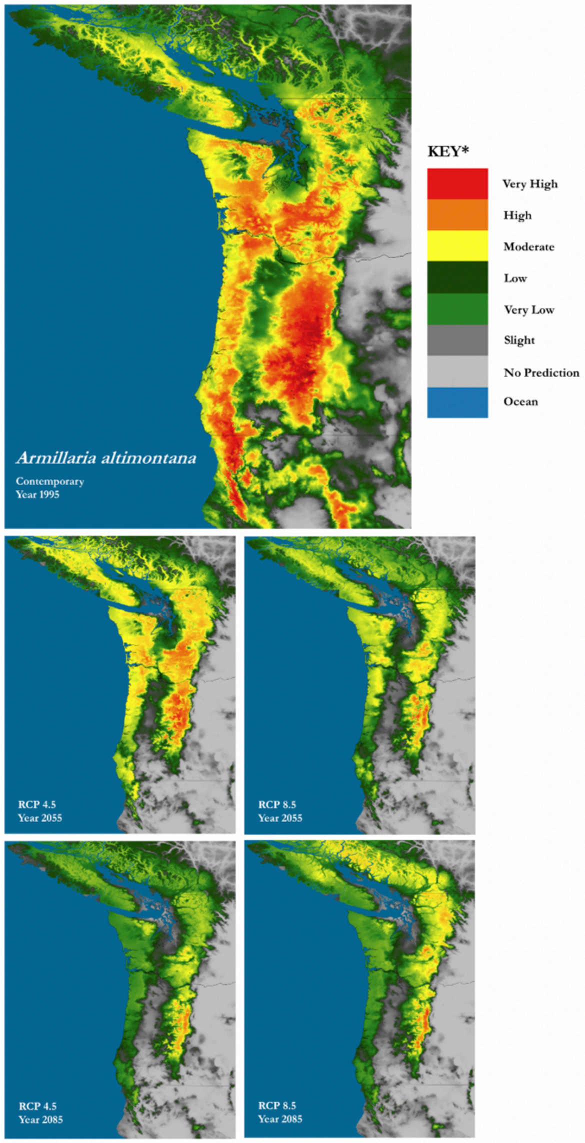
## Methods

Roots of trees and shrubs as well as basal portions of tree trunks were examined, and samples (e.g., rhizomorphs, mycelial fans, rotten wood) of *Armillaria* species were collected along with precise location information and associated environmental data. Cultured *Armillaria* isolates from western Washington, western Oregon, and northern California were then identified based on DNA sequence-based methods (e.g., translation elongation factor 1 alpha gene; *tef1*; Ross-Davis et al. 2012, Elías-Román et al. 2013, Klopfenstein et al. 2017). Over 200 isolates were collected from 2016 to 2018 and many additional isolates (ca. 100 that had been archived and curated by James B. Donley at the RMRS Forest Pathology Laboratory in Moscow, ID, USA) were re-evaluated after long-term storage at -80˚C from the USDA Forest Service-RMRS forest fungi collection in Moscow, Idaho (Zambino 2004, Ashiglar et al. 2014). These older isolates had been collected by Dave Shaw (1989) and Geral McDonald (1989-2003). These archived *A. solidipes* collections and three additional collections from southern British Columbia (location information was available in GenBank) were identified with IGS-1 sequence data (Kim et al. 2006). *Armillaria solidipes* IGS-1 data has been found to be adequate for its positive identification within the region, but it is less reliable for the other five *Armillaria* species found in the region (Kim et al. 2006, Hanna et al. 2007b).

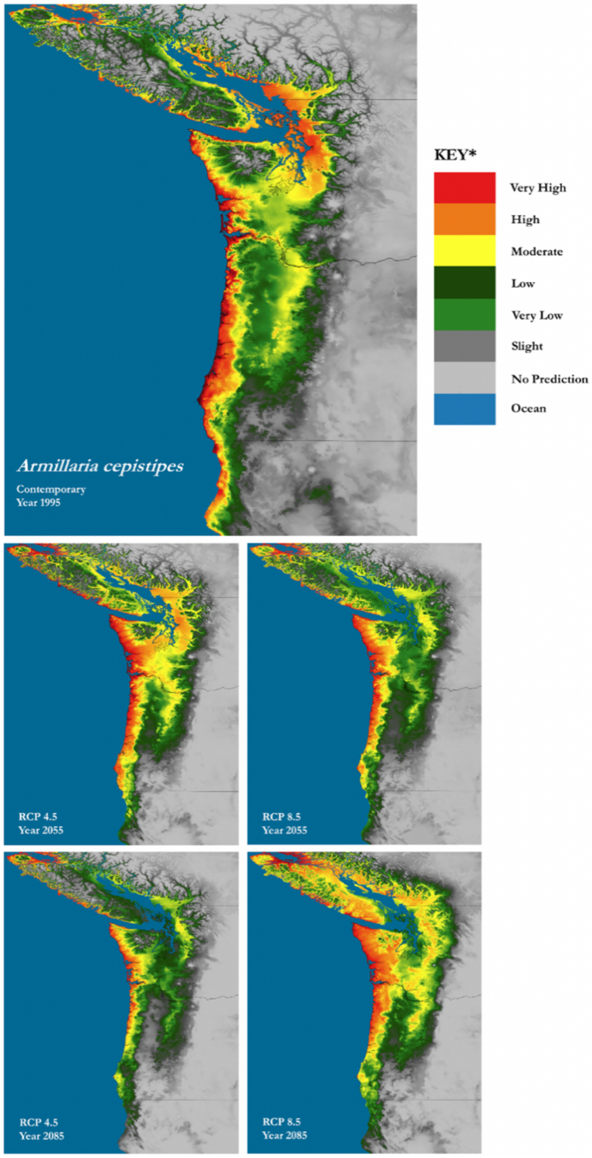
***Figure 1a:*** *Armillaria altimontana.*



Using these positively identified *Armillaria* isolates, a dataset of coordinates was created for use in bioclimatic modeling. Duplicate isolates of the same *Armillaria* species were eliminated if the geographic coordinates of their origin was within 0.25 km. Based on this reduced isolate set, a total of 13 locations for *A. altimontana*, 13 locations for *A. cepistipes*, 35 locations for *A. gallica*, 13 locations for *A. nabsnona*, 37 locations for *A. sinapina*, and 27 locations for *A. solidipes* were used for bioclimatic modeling. Locations used for modeling are denoted as small black dots on the maps (Figure 1a-f).

Bioclimatic models for each *Armillaria* species were created using Maxent software (Phillips et al. 2006). Input data for Maxent calculations in suitability models consisted of "samples with data" (SWD) files for each species that linked climate variable values for each of 26 bioclimatic variables with geographic coordinates (presence point localities). Additional input into Maxent included sets of interpolation grids (ca. 1-km$^2$ resolution) for current and projected climate data for North America (CMIP5 scenarios) provided by the AdaptWest Project (Wang et al. 2016). These climate data grids consisted of a set based on contemporary climate normals for the year 1995 (an average from 1981-2010) and two future sets for year 2055 (average from 2041-2070) and year 2085 (average from 2071-2100). For the future climate data, we chose a single

***Figure 1b:*** *Armillaria cepistipes.*



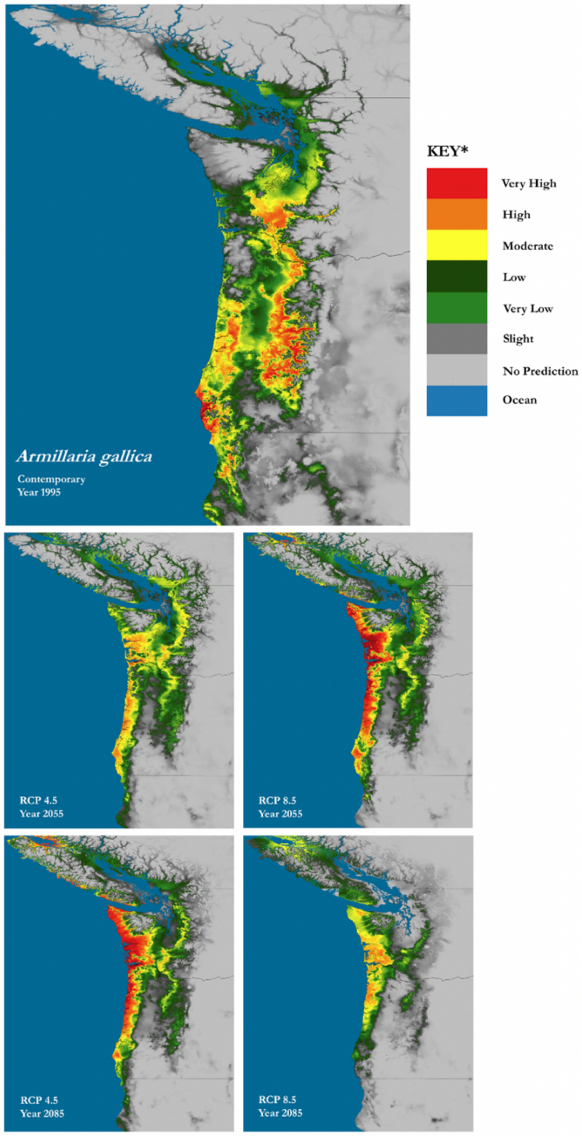
atmosphere-ocean general circulation model (AOGCM), the Hadley Global Environment Model 2 - Earth System (HadGEM2-ES), rather than an ensemble of dissimilar models (Bellouin et al. 2007, Collins et al. 2011, Martin et al. 2011). HadGEM2-ES is one of the eight available datasets from the AdaptWest Project; it fits in the middle of climate model genealogy for CMIP5 (performs similarly to ensemble models) and has been shown to be a top performer for the Pacific Northwest USA (Knutti et al. 2013, Rupp et al. 2013, Wang et al. 2016). The gridded climate layers were unprojected from Lambert Conformal Conic to an acceptable format for Maxent in degrees of latitude and longitude and then clipped to 28.5˚ W, 40˚ N, 120˚ W, 51˚ N so that random background points selected by Maxent would be within the study area of *Armillaria* locations. In all, grids for 26 biologically relevant variables, including seasonal and annual means, extremes, growing and chilling degree days, snow fall, potential evapotranspiration, and several drought indices were used for modeling.

Additional settings for each Maxent model included 10 replicates with the bootstrapping method (sampling with replacement) and a 25% test percentage. “Random seed” was also selected. Maxent uses background locations (pseudo-absences) to train the models. Background points for each model were created from 10,000 randomly selected geographical locations within the geographic range of the collected isolates. Maxent’s cumulative output (an index of probability from 0 to 100) was chosen for easier conceptualization compared to Maxent’s raw exponential model. A model for each *Armillaria* species was created for the contemporary time frame (year 1995) and then projected to future time periods (year 2055 and year 2085) using two different representative concentration pathways (RCPs – see Figure 2). This combination of six *Armillaria* species, a contemporary time period, two future time periods using two different RCPs produced 30 different outcomes (Figure 1a-f).

## Results and Discussion

Six *Armillaria* species from western Oregon and western Washington were identified: *A. solidipes*, *A. sinapina*, *A. gallica*, *A. nabsnona*, *A. cepistipes*, and *A. altimontana*. These *Armillaria* species appeared to have a range of ecological roles from pathogen to saprophyte. Generally, *A. solidipes* is known as an aggressive pathogen of conifers that causes significant growth loss and mortality; whereas, *A. gallica*, *A. sinapina*, *A. nabsnona*, *A. cepistipes*, and *A. altimontana* have been commonly characterized as primarily saprophytic with weak/variable pathogenicity (Gregory et al. 1991). However, recent observations suggest

***Figure 1c:*** *Armillaria gallica.*



that these less aggressive species can also be associated with root disease and/or deteriorating forest health, which frequently results in tree mortality (e.g., Dettman & van der Kamp 2001, Hanna et al. 2007a, Klopfenstein et al. 2009b, Kim et al. 2010, Cleary et al. 2012, Nelson et al. 2013, Klopfenstein et al. 2014, Burns et al. 2016, Kim et al. 2017, personal observations - J. W. Hanna).

Of the six *Armillaria* species, a low number of occurrence records was obtained for three species (*A. altimontana*, *A. cepistipes*, and *A. nabsnona*), with 13 occurrence records for each species; however, Maxent has been shown to be one of the best performing models for low numbers of presence records (Wisz et al. 2008). Pearson et al. (2007) had successful results with as few as five occurrence records, while Proosdij et al. (2016) indicates 13 occurrence records as a minimum to model "widespread" species. Nevertheless, our models performed well according to the AUC statistics. AUC values 0.5 to 0.6 represent a failed model, while 0.6 to 0.7 is poor, 0.7 to 0.8 is fair, 0.8 to 0.9 is good, and 0.9 to 1 is excellent model performance (Swets 1988). The following AUC values were obtained for the species models: *A. altimontana* = 0.896 (good), *A. cepistipes* = 0.922 (excellent), *A. gallica* = 0.965 (excellent), *A. nabsnona* = 0.895 (good), *A. sinapina* = 0.934 (excellent), and *A. solidipes* = 0.937 (excellent). The predicted species distribution range indicates that *A. cepistipes* and *A. nabsnona* are perhaps more widespread than previously reported (Banik et al. 1996, Volk et al. 1996). The projected future distributions for *A. cepistipes* and *A. nabsnona* also show predicted occurrences along the coastal areas. *Armillaria gallica* and *A. sinapina*, the two most commonly found species in our study, show substantial reductions in predicted climatically suitable areas under the projected future-climate scenarios. If *A. sinapina* cannot adapt to the predicted future climate, it may become rare or extirpated in the region by the year 2085. Likewise, the prediction for *A. altimontana* shows a substantial reduction in predicted range by the year 2085. Noteworthy is that *A. altimontana* has been shown to have potential benefits to growth and survival of western white pine (*Pinus monticola*) in north Idaho and exerts potential biocontrol against the pathogenic species, *A. solidipes* (Warwell et al. 2019). As for predictions for *A. solidipes* "the most aggressive destroyer of forests*," suitability decreases in its southern range but increases in the north by the year 2085 (* quote from Phil Cannon). The information from these models will contribute to management strategies for reducing detrimental impacts and increasing the benefits of these ecologically important fungal species.

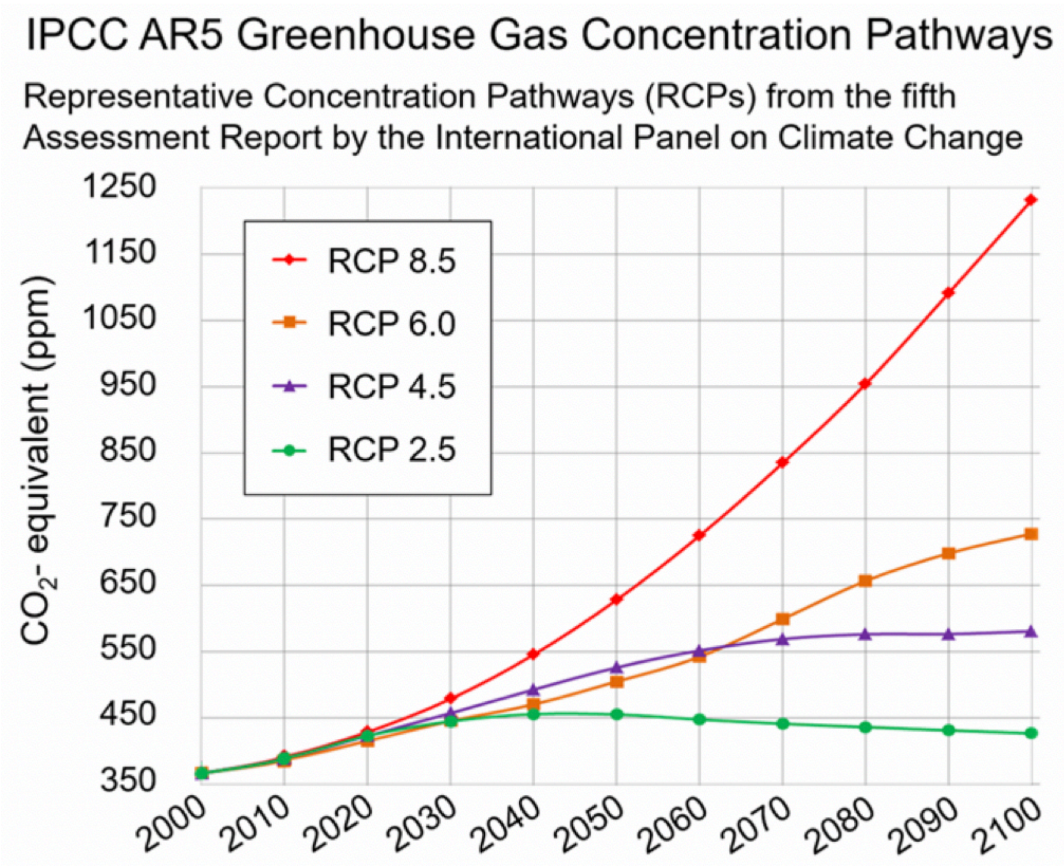


***Figure 2:*** *Differences in RCPs (Representative Concentration Pathways) 4.5 and 8.5 for future time periods. RCPs are greenhouse gas concentration (not emissions) trajectories adopted by the IPCC for its fifth Assessment Report (AR5) in 2014. It supersedes Special Report on Emissions Scenarios (SRES) projections published in 2000. In general terms, RCP 8.5 represents a rampant increase in fossil fuel energy use by the end of the century, and no mitigation to control* $CO_2$ *concentrations. While, RCP 4.5 represents a future where energy use increases; however, there is a substantial growth in renewable energy resources by the year 2050. "The Beginner's Guide to Representative Concentration Pathways" by G.P. Wayne provides informative details. https://skepticalscience.com/docs/RCP_Guide.pdf.*

## Future Work

In addition to acquiring more occurrence points for *A. altimontana*, *A. cepistipes,* and *A. nabsnona*, studies on predicted *Armillaria* distributions could be improved by obtaining population-level data to run independent predictions based on separate populations. Differing populations within species may be adapted to differing climatic niches. These studies are also critically limited by the dearth of *Armillaria* distribution data from California. *Armillaria* species in California are likely adapted to climates that relate to the future climate scenarios for the Pacific Northwest, USA. Location data based on current methods to identify *Armillaria* species in California are unavailable, because it has been nearly 20 years since the distribution of *Armillaria* in California was published (Baumgartner & Rizzo 2001). Finally, predictions of future climates are continually improving and new atmosphere-ocean general circulation models and representative concentration pathways (or similar scenarios) will improve the models based on predicted future climates. For example, data not included in current estimates of climate change (e.g. currently available climate grids/surfaces; Wang et al. 2016, Fick & Hijmans 2017, and others) include invasive earthworms in the boreal forests that change global carbon dynamics (Lubbers et al. 2013) and increased $CO_2$ sensitivity with increasing temperatures (Friedrich et al. 2016). In addition, our predictions are based on climate averages; however, increases in the occurrence and intensity in extreme climate events will also likely impact species survival and distribution (Rahmstorf & Coumou 2011).

***Figure 1d:*** *Armillaria nabsnona.*

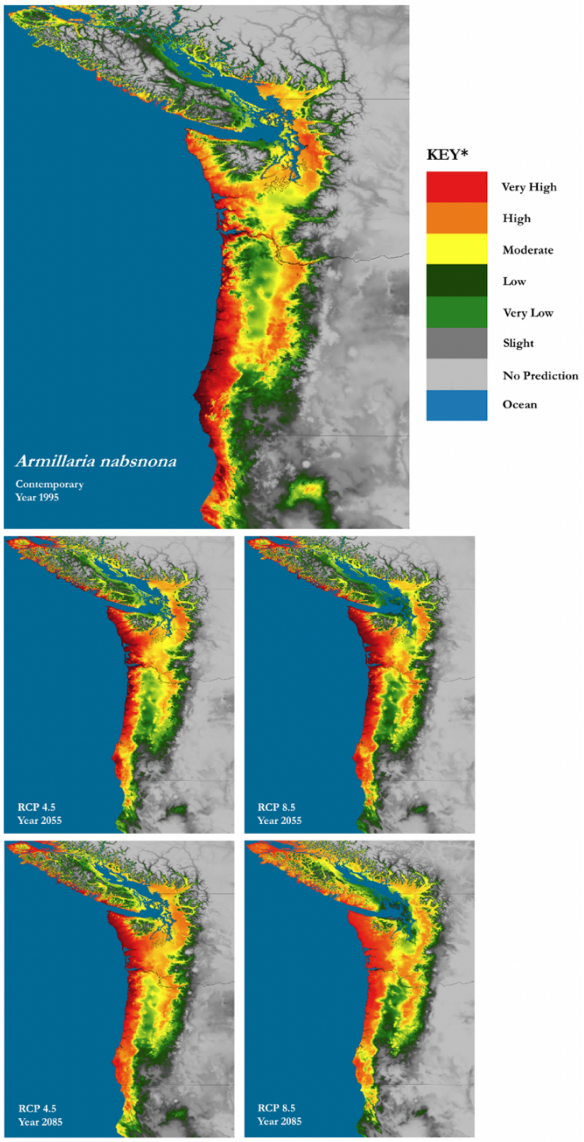


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***Figure 1e:*** *Armillaria sinapina.*

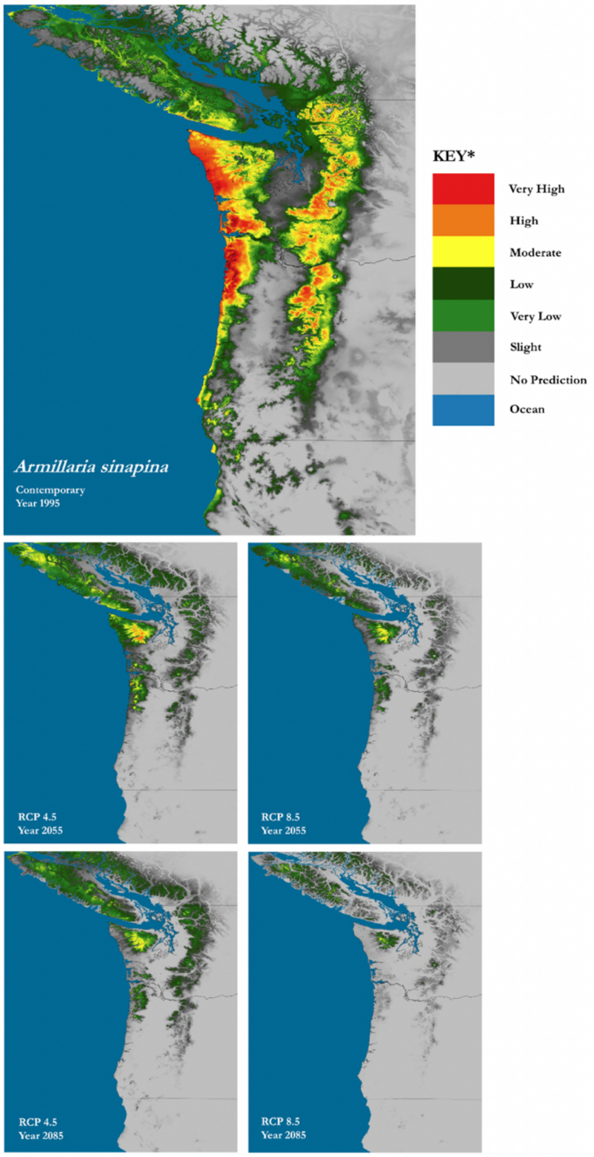

***Figure 1f:*** *Armillaria solidipes.*

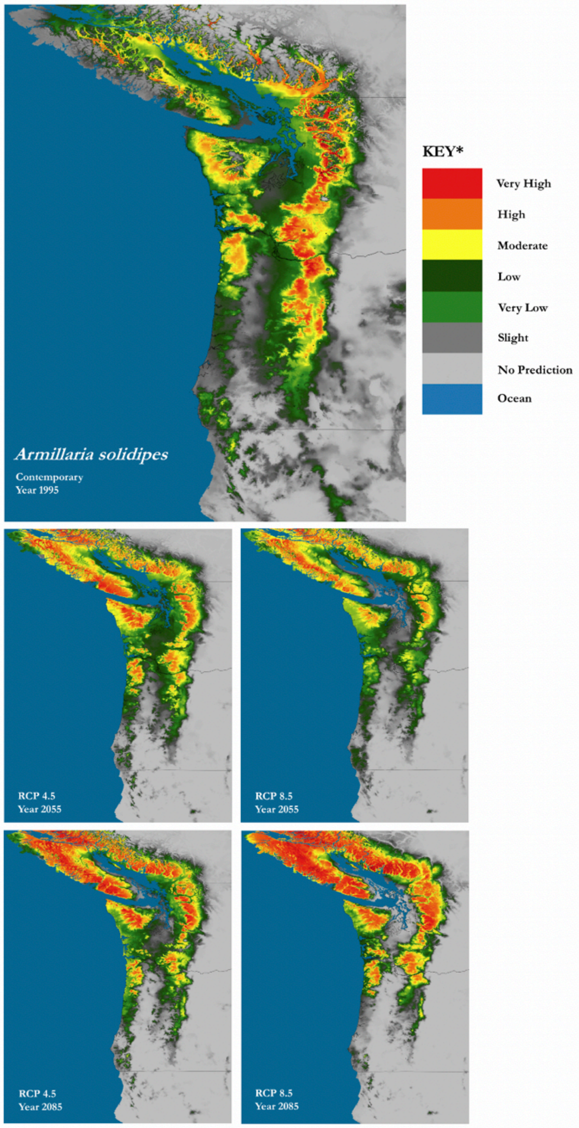

***Figure 1a-f:*** *Maximum Entropy bioclimatic model of suitable climate space (potential distribution) for six* Armillaria *species. Largest map on top is a model of suitable climate space based on climate normals from 1981 to 2010. Smaller sets of four future models show two different RCPs [Representative concentration pathways, RCP4.5 and RCP8.5 (see Figure 2)] for the years 2055 and 2085. Darkest gray represents predicted suitable climate space, with light green, yellow, orange, and red indicating increased suitability, respectively. Small black dots represent collection locations for corresponding* Armillaria *species maps that were used to produce the models. Listed years represent averages, 1995 = climate normals from years 1981-2010, 2055 = years 2041-2071, 2085 = years 2071-2100.*

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# BIOCLIMATIC MODELING OF *ARMILLARIA* SPECIES IN SOUTHEASTERN ALASKA, INCLUDING POTENTIALLY INVASIVE *ARMILLARIA* SPECIES UNDER CHANGING CLIMATE

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## Introduction

Armillaria root disease, caused by several *Armillaria* species, is one of most damaging tree diseases throughout the world (Wargo & Shaw 1985). In southeastern Alaska, both *A. sinapina* and *A. nabsnona* have previously been reported, but these species have generally been considered as weak pathogens or saprophytes under natural conditions in this region (Shaw & Loopstra 1988, Adams et al. 2015). More recently, however, *A. sinapina* has been found in pathogenic situations in Alaska (Klopfenstein et al. 2009a, Adams et al. 2015). In Shaw and Loopstra's (1988) study, they also identified two *Armillaria* spp. (*A. sinapina* and *A. nabsnona*) in addition to unknown *Armillaria* sp. from Alaska, which may represent new species. With the discovery of active and potentially increasing Armillaria root disease in Alaska and the possibility of new *Armillaria* species in the region, we investigated potential *Armillaria* species distributions using DNA sequence-based identification coupled with bioclimatic modeling (Klopfenstein et al 2009b, Ross-Davis et al. 2012, Elías-Román et al. 2013, and Klopfenstein et al. 2017).

For bioclimatic modeling for southeastern Alaska, we used point locations from the North American Pacific Northwest (southwestern British Columbia, western Washington, western Oregon, northern California) under the future climate scenario of RCP 8.5 for the year 2085. These models show potential invasive threats posed by *Armillaria* from the Pacific Northwest, should these species become established in southeastern Alaska. While non-native *Armillaria* species have yet to be reported as invasive in North America, reports of European/Asian *A. mellea* and *A. gallica* are documented in the literature for South Africa (Coetzee et al. 2001, Coetzee et al. 2003, Mwenje et al. 2006, Wingfield et al. 2010). Inoculum could obviously arrive via natural migration or on woody material infected with rhizomorphs or mycelial fans, which could potentially arrive undetected at ports of entry. Furthermore, introduction of basidiospores, which have been shown to survive on tree bark through an Alaskan winter (Shaw 1981), would not be readily detectable with current technologies.

## Objectives

1. Isolate, culture, and identify *Armillaria* species from surveys/sample collections from georeferenced locations in southeastern Alaska
2. Predict current suitable climate space distribution of all species identified in the region
3. Project these distributions for future time periods under several climate change scenarios
4. Predict potentially invasive *Armillaria* species based on modeled contemporary suitable climate for *Armillaria* species of the Pacific Northwest and predicted future climate in southeastern Alaska

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## Methods

Major roots and butts of trees and shrubs were examined, and samples (i.e., rhizomorphs, mycelial fans, rotten wood) of *Armillaria* species were collected along with precise location information and associated environmental data. Cultured *Armillaria* isolates from southeastern Alaska were then identified based on current DNA sequencing methods (e.g., translation elongation factor 1-alpha: *tef1* gene) as described (Ross-Davis et al. 2012, Elías-Román et al. 2013, and Klopfenstein et al. 2017). Forty-nine collections were made from 2016 to 2018. From positively identified isolates, a dataset of coordinates was created for use in bioclimatic modeling. Duplicate species isolates collected within 0.25 km were eliminated from analyses. After these isolates were eliminated from the analyses, 12 locations for *A. sinapina* were obtained for bioclimatic modeling (Figure 1). A single isolate of *A. nabsnona* was identified from Prince of Wales Island, but this was insufficient for bioclimatic modeling (Figure 2. *A. nabsnona* map). *Armillaria* locations from the Pacific Northwest were used to model potentially invasive *Armillaria* species for this region. For details about the Pacific Northwest isolate numbers and locations used in modeling, please refer to another WIFDWC proceeding paper by Hanna et al. (Maximum entropy-based bioclimatic models predict areas of current and future suitable habitat for *Armillaria* species in western Oregon and western Washington) in this volume.

Models for each *Armillaria* species were created using Maximum Entropy (Maxent) software (Phillips et al. 2006). Input data for Maxent calculations in suitability models consisted of “samples with data” (SWD) files for each species that linked climate variable values for each of 26 bioclimatic variables with geographic coordinates (presence point localities). Additional input into Maxent included sets of interpolation grids (ca. 1-km$^2$ resolution) for current and projected climate data for North America (CMIP5 scenarios) provided by the AdaptWest Project (Wang et al. 2016). These consisted of a set based on contemporary climate normals for the year 1995 (an average from 1981-2010) and two future sets for year 2055 (average from 2041-2070) and year 2085 (average from 2017-2100). For the future data, we chose a single atmosphere-ocean general circulation model (AOGCM), Hadley Global Environment Model 2 - Earth System (HadGEM2-ES), rather than an ensemble of dissimilar models (Bellouin et al. 2007, Collins et al. 2011, Martin et al. 2011). HadGEM2-ES is one of the eight available datasets from the AdaptWest Project; it fits in the middle of climate model genealogy for CMIP5 (performs similarly to an ensemble) and has been shown to be a top performer for the Pacific Northwest USA (Knutti et al. 2013, Rupp et al. 2013, Wang et al. 2016). The gridded climate layers were unprojected from Lambert Conformal Conic to an acceptable format for Maxent in degrees of latitude and longitude and then clipped to 141° W, 40° N, 120° W, 60° N so that random background points selected by Maxent would be located within the study area of *Armillaria* locations, which is different than the approach used by Hanna et al. (2019). In all, grids for 26 biologically relevant variables, including seasonal and annual means, extremes, growing and chilling degree days, snow fall, potential evapotranspiration, and several drought indices were considered.

Additional settings for each Maxent model included 10 replicates with the bootstrapping method (sampling with replacement) and a 25% test percentage. “Random seed” was also selected. Maxent uses background locations or pseudo-absences to train the models. Background points for each model were created from 10,000 randomly selected geographical locations within the geographic range of the collected isolates. Maxent’s cumulative output (an index of probability from 0 to 100) was chosen for easier conceptualization compared to Maxent’s raw exponential model. Two separate contemporary (year 1995) models (and four corresponding future projections for each – year 2055 and 2085 x RCP 4.5 and RCP 8.5) based on the 12 *A. sinapina* locations from southeastern Alaska and based on *A. sinapina* locations from the Pacific Northwest are shown in Figure 1. To investigate potential invasiveness of *Armillaria* species into southeastern Alaska, the same future-climate models were run for the five other species locations of the Pacific Northwest, but only year 2085 – RCP 8.5 (Figure 2).

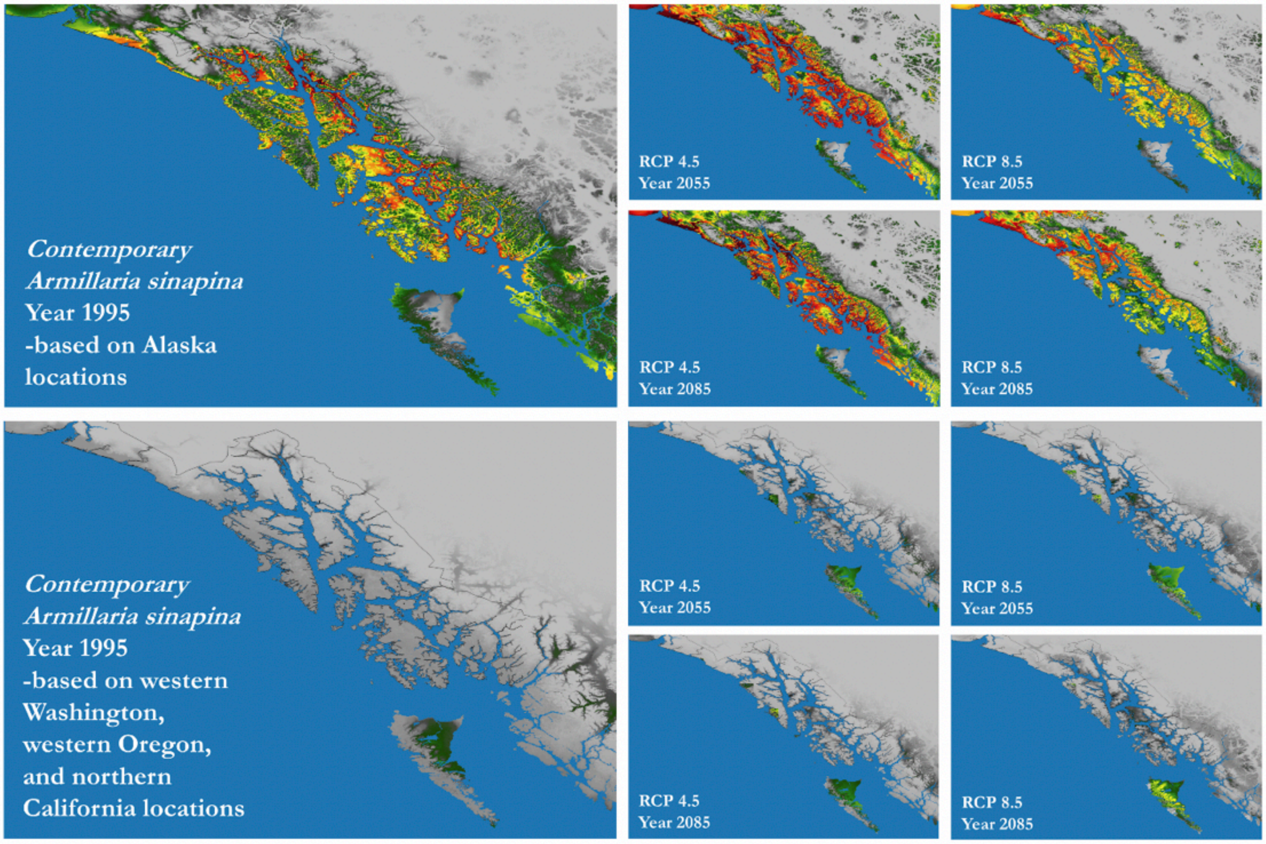
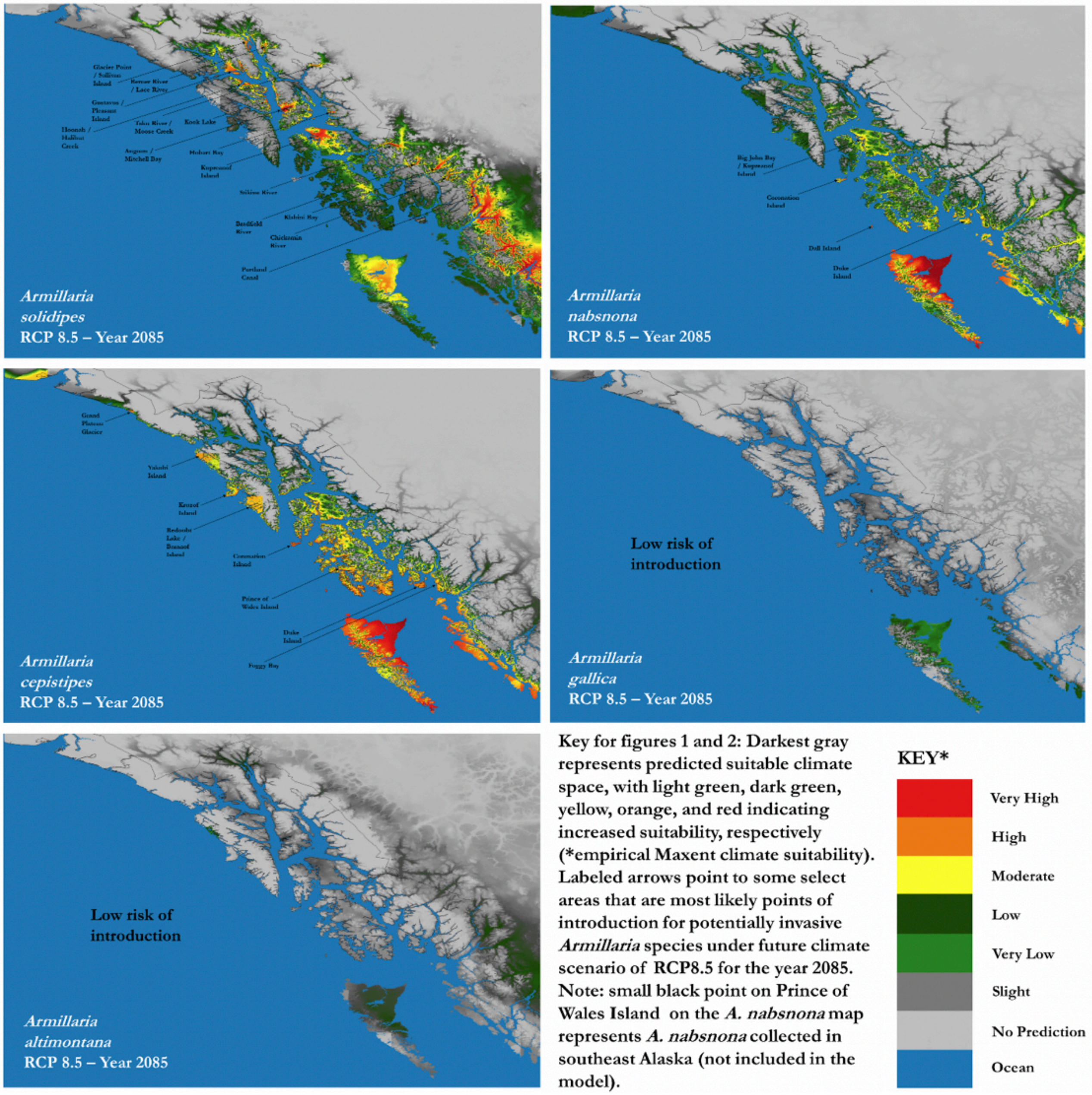


***Figure 1:*** *Modeled suitable climate for* Armillaria sinapina *based on southeastern Alaska locations (top) versus based on northern California, western Oregon, and western Washington locations (bottom). Does this show populations adapted to different climate niches or does* A. sinapina *have a great ability to adapt to dissimilar climates? (Note: see Figure 2 for KEY).*

## Results and Discussion

Our surveys from 2016 – 2018 in southeastern Alaska yielded 49 sample collections. Using DNA sequences of the *tef1* gene, 32 isolates were confirmed as *A. sinapina* and one isolate as *A. nabsnona*. This result confirms previous surveys that showed these *Armillaria* species are present in southeastern Alaska (e.g., Klopfenstein et al. 2009a, Shaw & Loopstra 1988).

Results were limited by the low number of occurrence locations for three of the six Pacific Northwest *Armillaria* species (13 occurrence locations for *A. altimontana*, *A. cepistipes*, and *A. nabsnona*) and only 12 occurrence locations for *A. sinapina* from southeastern Alaska. However, Maxent has been shown to be one of the best performing models based on limited presence data (Wisz et al. 2008). Pearson et al. (2007) had successful results with as few as five occurrence records while Proosdij et al. (2016) indicated 13 occurrence records as a minimum to model "widespread" species. AUC [Area Under the ROC (Receiver Operating Characteristics) Curve] values 0.5 to 0.6 represent a failed model while 0.6 to 0.7 is poor, 0.7 to 0.8 is fair, 0.8 to 0.9 is good and 0.9 to 1 is excellent (Swets 1988). The AUC performance measure of the model from the 12 southeastern Alaska *A. sinapina* locations had a value of 0.988 (excellent). The Pacific Northwest model AUC values were *A. altimontana* = 0.970 (excellent), *A. cepistipes* = 0.973 (excellent), *A. gallica* = 0.986 (excellent), *A. nabsnona* = 0.969 (excellent), *A. sinapina* = 0.977 (excellent), and *A. solidipes* = 0.981 (excellent).



Key for figures 1 and 2: Darkest gray represents predicted suitable climate space, with light green, dark green, yellow, orange, and red indicating increased suitability, respectively (*empirical Maxent climate suitability). Labeled arrows point to some select areas that are most likely points of introduction for potentially invasive *Armillaria* species under future climate scenario of RCP8.5 for the year 2085. Note: small black point on Prince of Wales Island on the *A. nabsnona* map represents *A. nabsnona* collected in southeast Alaska (not included in the model).

***Figure 2:*** *Potential* Armillaria *species invasiveness based on a relatively high climate-change scenario (RCP8.5 for the years 2085). Bioclimatic model predictions are based on current* Armillaria *occurrences in southern British Columbia, western Oregon, and western Washington*.

Based on our model results, *A. sinapina* in southeastern Alaska appears to exist under significantly different climatic conditions than *A. sinapina* in the Pacific Northwest (Figure 1). *Armillaria sinapina* from the Pacific Northwest does not appear to have much suitable climate space in the southeastern Alaska region even when we look at the high climate-change scenario (RCP 8.5 for the year 2085). At this point, we can only speculate as whether these results indicate that *A. sinapina* comprises different populations that are adapted to different climates or a high adaptability for climate within *A. sinapina*. Also, of note is climatic conditions in southeastern Alaska are predicted to greatly expand suitable climate space for *A. sinapina* by the year 2055 under the RCP 4.5 scenario, and this trend continues into 2085. Under the RCP 8.5 scenario, suitable climate space for *A. sinapina* is reduced in the south but increased in the north.

Based on the modeling of *Armillaria* species locations from the Pacific Northwest, the models show *A. nabsnona*, *A. cepistipes*, and *A. solidipes* all appear to have locations with suitable climate for survival in southeastern Alaska by the year 2085. And, perhaps unfortunately, we predict that future suitable climate space for *A. altimontana*, a potential biocontrol against *A. solidipes* (Warwell et al. 2019), will not exist within this region under the projected future climate scenario. Adding to this scenario, host trees may also be more susceptible to pathogenic *Armillaria* spp. due to maladaptive stress in a new environment under changing climate.

## Acknowledgements

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# TOWARD DNA-BASED AND BIOCLIMATIC MODELING TOOLS TO IDENTIFY *ARMILLARIA* PATHOGENS, PREDICT HOST/PATHOGEN DISTRIBUTIONS, AND MANAGE ARMILLARIA ROOT DISEASE IN THE INTERMOUNTAIN REGION (USA) UNDER CHANGING ENVIRONMENTS

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## Introduction

Armillaria root disease is one of the leading causes of growth loss and mortality in coniferous forests of the western USA (Lockman & Kearns 2016). Recent surveys suggest Armillaria root disease is increasing on trees in Utah (John Guyon, per. comm.). To maintain forest resilience and productivity, we must determine (1) which *Armillaria* species are involved in current root diseases and associated mortality in forests across the region, (2) where these *Armillaria* species occur/will occur across the current and future landscapes, and (3) where changing climates could exacerbate this disease. Previous studies suggest that Armillaria root disease is more severe in trees that are maladapted to climate-induced stress (e.g., Klopfenstein et al. 2009, Kliejunas et al. 2009, Sturrock et al. 2011, Dempster 2017, Kubiak et al. 2017, Aslam & Magel 2018). Thus, it is likely climate change will further exacerbate damage from Armillaria root disease.

Although Armillaria root disease is known to occur in the Intermountain Region (USDA Forest Service Region 4) of the USA, only *A. solidipes* (previously *A. ostoyae*) was identified within the region (McDonald 1999). Based on recent data and data from neighboring regions, other *Armillaria* species also occur in Region 4, including *A. sinapina*, *A. cepistipes*, *A. gallica*, and *A. altimontana* (Blodgett & Lundquist 2011, Blodgett et al. 2015, RMRS collections, Figure 1). Additional information for these species is needed to predict current and future distributions and associated threats to tree health (Figures 2 and 3). With newly acquired data, we can use DNA-based methods to identify *Armillaria* species, and bioclimatic models to predict pathogen and host distributions across the region (Klopfenstein et al. 2009, Figure 4).

Data from this project will be incorporated into existing bioclimatic models from other regions in western North America (USDA Forest Service Regions 2, 3, 6, and 10) to build west-wide bioclimatic models for *Armillaria*. This will improve the model accuracy and expand the geographic extent. These tools predict geographic areas where climate change is predicted to exacerbate Armillaria root disease and can predict areas where tree species that are tolerant to Armillaria root disease will be climatically adapted.

## Objectives

The objectives of this project are to: (i) identify *Armillaria* species from collections/surveys of under-represented areas in the Intermountain Region; and (ii) use the occurrence data to evaluate and refine bioclimatic models for predicting the present and future suitable climate space (potential distribution or the geographic area that is climatically suitable for a species' survival). New *Armillaria* species/host combinations within the region will be documented.

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To supplement under-represented areas, additional *Armillaria* surveys/collections are needed in the Intermountain Region (Figure 1). We have asked collaborators to survey for *Armillaria* within these areas and send us *Armillaria* samples (e.g., mycelial fans on live trees indicating disease activity or rhizomorphs) along with GPS information and host/environmental information (Figure 3).

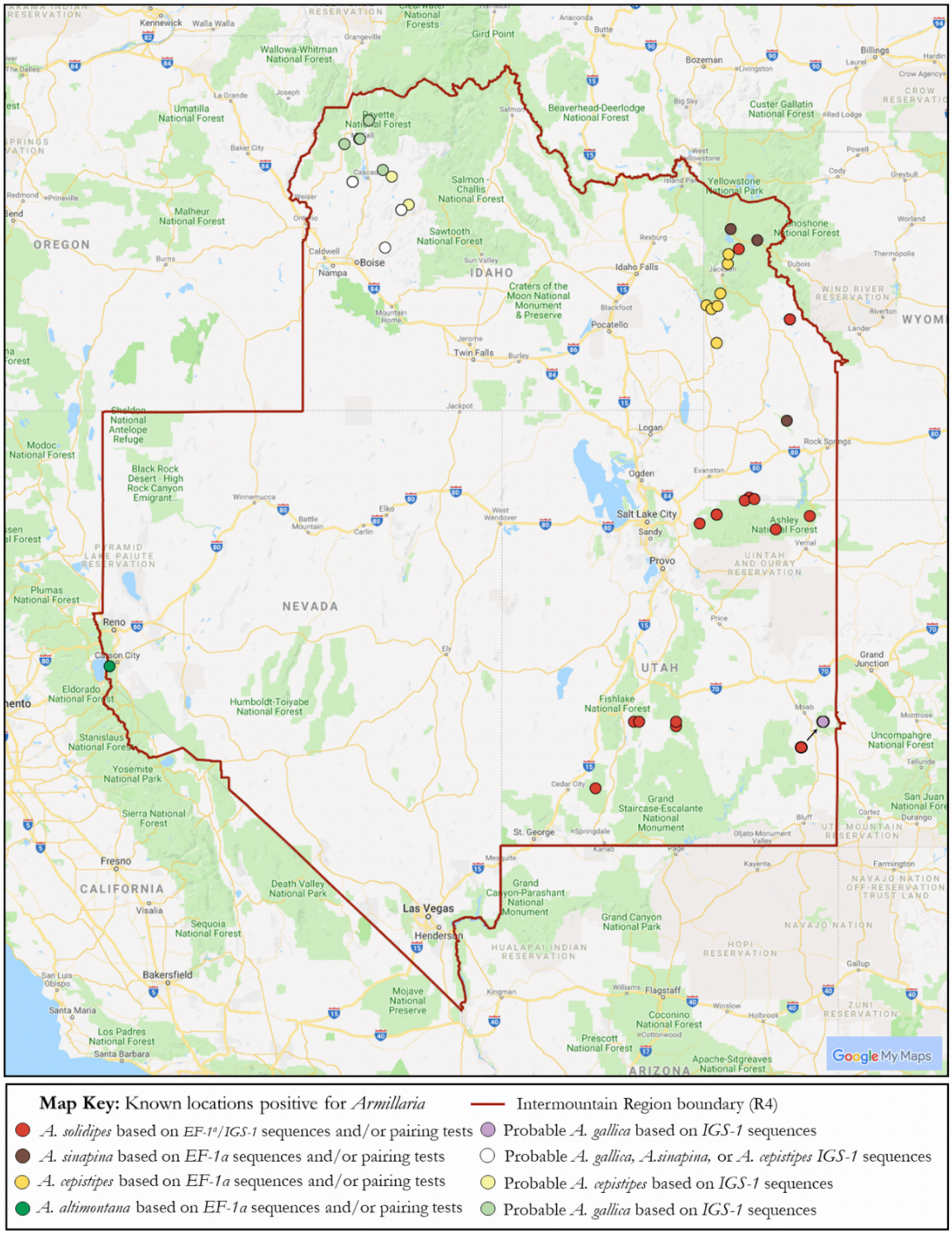

***Figure 1:*** *USDA Forest Service Intermountain Region (R4) - Study area.*

***Figure 2:*** Armillaria *surveys. Armillaria root disease center (upper left); Excavating a root collar to survey for* Armillaria *(upper right);* Armillaria *fruiting bodies or basidioma (lower left);* Armillaria *rhizomorphs (lower center); and* Armillaria *mycelial fan (lower right).*

## Methods

Conduct DNA sequence-based diagnostics to identify *Armillaria* species collected from across the Intermountain Region: Primary root systems and butts of trees and shrubs will be examined, and samples (e.g., rhizomorphs, mycelial fans, rotten wood) of *Armillaria* spp. collected along with precise location and associated environmental data (Figures 2 and 3). Cultured *Armillaria* isolates from each site will be identified based on DNA sequencing (Kim et al. 2006, Hanna et al. 2007, Ross-Davis et al. 2012, Klopfenstein et al. 2017). Sampling locations will initially be selected based on information of Armillaria root disease from FHP personnel, and other areas with vegetation that could support *Armillaria* spp.

***Figure 3:*** Armillaria *sample collection. GPS location (left), labeled collection bag for mycelial fans and/or infected wood (center); and labeled tubes for rhizomorphs (right).*

*Armillaria* survey data will be integrated into a bioclimatic model to predict suitable climate space (potential distribution) across the Intermountain Region. A bioclimatic model, such as MaxEnt (Phillips et al. 2006), will be used to determine which climatic factors contribute to the occurrence of *Armillaria* spp. across the landscape. Future distributions of suitable climate space for *Armillaria* will be predicted based on various General Circulation Models and greenhouse gas-emission scenarios (Klopfenstein et al. 2009, Hanna et al. 2016), and compared with present realized niche and its predicted distribution under future climate scenarios for many host tree species, which have already been predicted (Rehfeldt et al. 2006). In addition, predictions will be updated with improved climate surfaces, as available. In general, potential distribution of *Armillaria* root disease will be predicted for areas where the pathogen is climatically adapted, with increased risk associated with areas where the host is climatically maladapted.

Bioclimatic data will be made available for incorporation into web-based, decision tools: Management recommendations will be provided to reduce Armillaria root disease for projected future climates (e.g., 2050, 2080). These decision-support tools will provide guidance for field units for project planning, NEPA documents, forest plan revisions, and show at risk habitat in regard to endangered species.

## Expected Outcomes and Benefits

Products from this project will guide forest management by producing decision-support tools that predict potential distribution of Armillaria root disease under present and future climate scenarios. Management recommendations will offer strategies to reduce Armillaria root disease for projected future climates (e.g., 2050s, 2080s, etc.) based on climatic adaptation of tree species in relation to *Armillaria*. This information can also be incorporated into National Disease Risk maps, which will help develop appropriate disease management protocols. In addition, this project can be readily adapted to other important forest diseases (endemic and invasive). We plan to share our products among the community of forest health professionals and share accompanying information via publications, presentations, on-site workshops, and online.

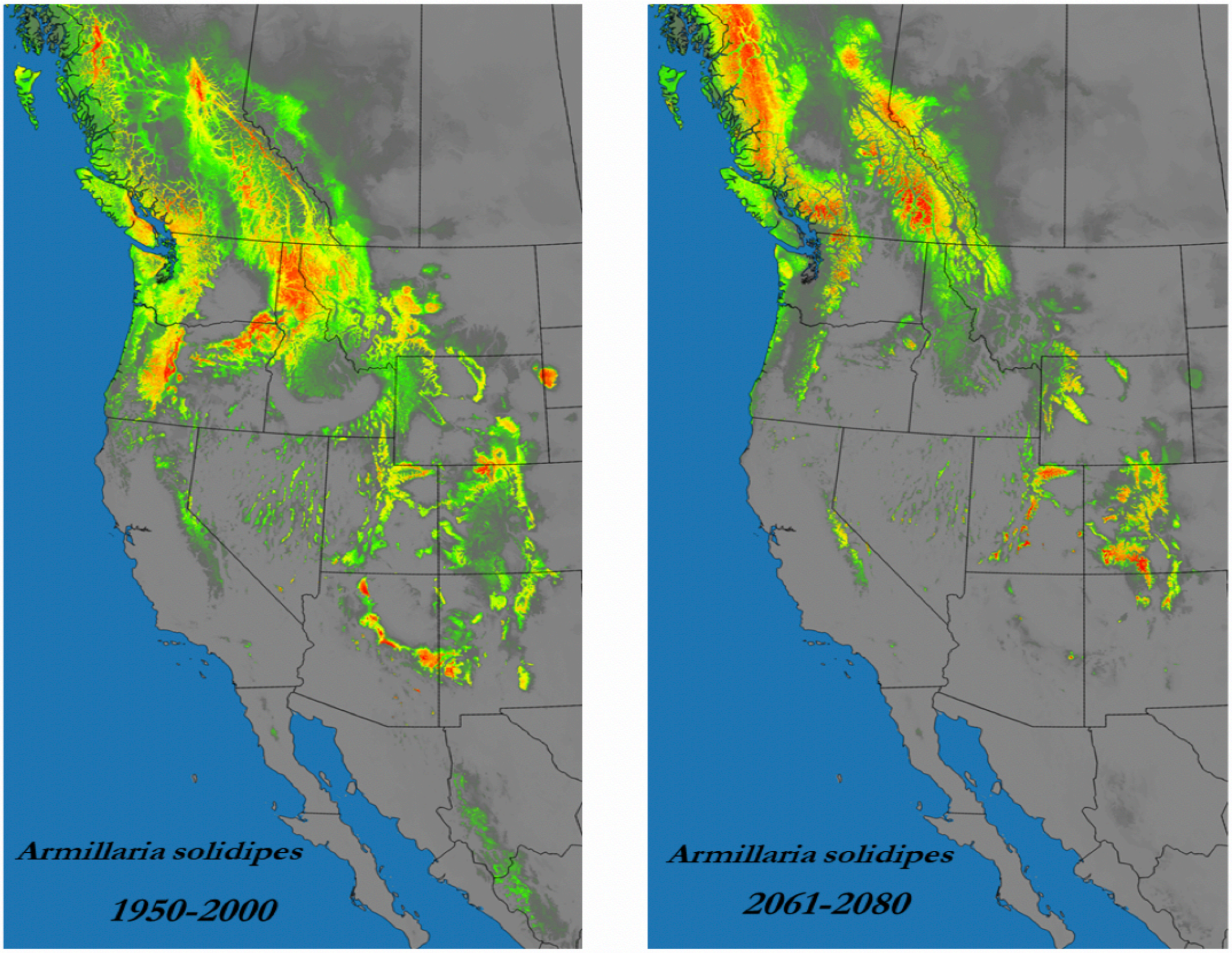

***Figure 4:** Preliminary example (based on limited data) Maximum Entropy bioclimatic model of realized climate niche for predicted contemporary* Armillaria solidipes *(left image) and predicted for the years 2061-2080* Armillaria solidipes *(right image). Darkest gray represents predicted suitable climate space, with light green, yellow, orange, and red indicating increased suitability, respectively.*

## Acknowledgements

This project is supported by USDA – Forest Service, State and Private Forestry, Forest Health Protection – Special Technology Development Program and Rocky Mountain Research Station – Forest Woodland Ecosystems Program.

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# DEVELOPMENT OF A LAMP ASSAY FOR THE CAUSAL AGENT OF WHITE PINE BLISTER RUST, *CRONARTIUM RIBICOLA*

**Jorge R. Ibarra Caballero[1], K.A. Leddy[1], and Jane E. Stewart[1]**

## Introduction

The fungus *Cronartium ribicola* causes the White Pine Blister Rust disease, considered one of the most destructive diseases of five-needled (white) pines (Maloy 2001). The disease has a life cycle that requires two hosts: a white pine and *Ribes* spp. (Maloy 2003), although other non-*Ribes* species have been demonstrated to act as alternate hosts as well (McDonald et al. 2006). Infected needles show indistinct chlorotic spots; later the infected branches develop slight swellings, but it can take years for the tree to produce cankers that later will produce distinct spores at the margin (Maloy 2003). On the alternate host, indistinct yellow spots appear on the upper surface of the leaves weeks after infection, followed by orange pustules on the underside of infected leaves. Early detection methods of infected hosts would be helpful for the surveillance and management of this disease. Recently, Bergeron et al. (2019), developed specific real time PCR assays for *C. ribicola* and other tree pathogens using a whole genome comparison approach. We used the regions on the *C. ribicola* genome where the Real Time PCR primers annealed to develop a loop mediated isothermal amplification (LAMP) specific assay for this pathogen. We also tested a simple extraction method from wood samples to prepare the DNA samples for the LAMP assay. The DNA extraction and LAMP assay take 30 minutes each and require relatively little investment in equipment. More testing is still required to fully validate this assay.

## Material and Methods

Genome sequence of *Cronartium ribicola* were obtained from the NCBI GeneBank, accesion AWVX00000000.1. Sequences of primer and probe for the real time PCR were obtained from Bergeron et al. (2019). BLASTn was used to map the primers to the genome to get the *C. ribicola* specific genome region. A larger region that encompasses the previous was used in the PrimerExplorer LAMP primer designer software (Fujitsu 2005). Oligonucleotides with the primers sequences were obtained from IDT (Iowa). WarmStart Colorimetric LAMP 2X Master Mix was obtained from NEB (Massachusetts). LAMP ready mix consisted of 0.2 µM each F3 and B3 primers, 0.4 µM each loopF and loopB primers, 1.6 µM each FIP and BIP primers, 1x WarmStart master mix and water for a total volume of 24 µl. DNA samples were added in 1 µl volume. Purified DNA was obtained from wood or spore samples using a modified CTAB method (Cubero et al. 1999). For Chelex extraction, Chelex 100 resin was obtained from BioRad (California). Approximately a 0.1 ml volume of wood was added to 200 µl of 5% Chelex solution, mixed and incubated for 30 minutes at 98-99 C (Figure 1); supernatant aliquots were used for the LAMP reaction. The reaction mix including the DNA sample was incubated at 65°C for 30 minutes. Results were assessed by the color change from pink (negative) to yellow (positive) (Figures 2 and 3).

## Results and Discussion

Primers for the LAMP assay were designed on a slightly larger region than the region used in Bergeron et al. (2019) (Figure 4); using the same region was important to get the same specificity. The LAMP assay

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has been tested with wood or leaf samples that were confirmed to be infected with *Cronartium ribicola*, *C. comandrae*, *Peridermiun bethelii,* or *Puccinia caricis* by sequencing of the ITS region. Of those samples only, ITS *C. ribicola* positive samples produced a positive result using the LAMP assay.

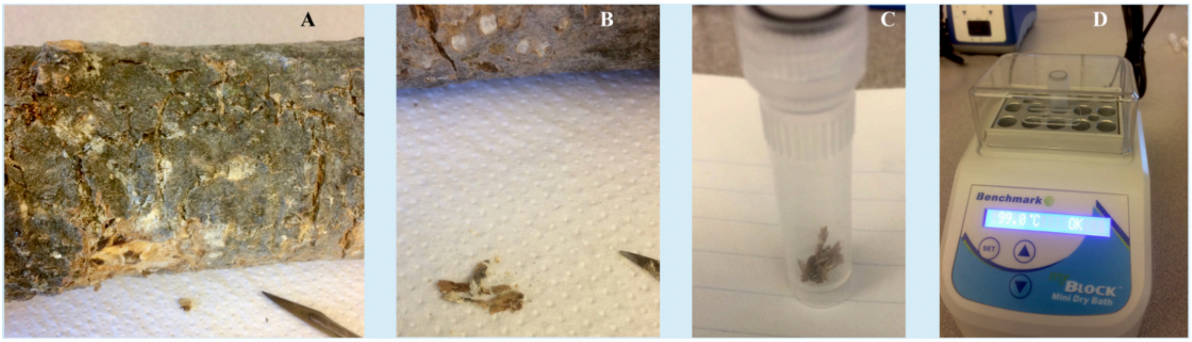

***Figure 1: A:*** *example of branch sample from where small pieces of wood were taken for DNA extraction* ***(B,C). D:*** *Incubation of tube with wood samples in chelex 100 5% in portable heat block.*

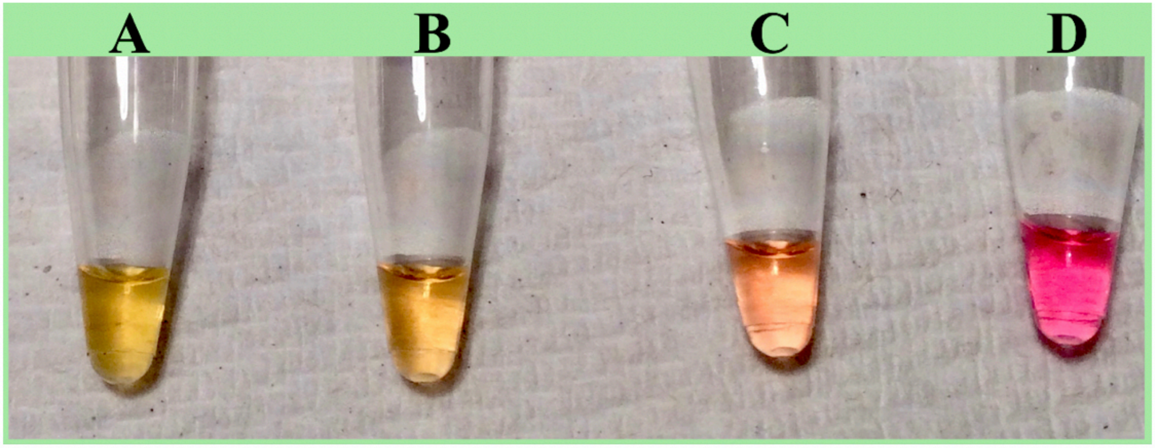

***Figure 2:*** *Ten fold dilutions of* C. ribicola *DNA LAMP assay. A: 1 ng; B: 100 pg; C: 10 pg; D: negative control.*

Tenfold serial dilutions of purified *C. ribicola* DNA were used to evaluate the sensitivity of the method. One ng, 100 pg and 10 pg produced a clear positive reaction although the lowest amount was slightly orange compared to the pink usually observed in a positive reaction (Figure 2).

To test the feasibility of using the LAMP assay away from laboratory facilities, corresponding volumes of WarmStart master mix and each one of the 6 used primers were pre-mixed and stored at 4°C for 24 hours or -20°C for 2 weeks. Results were the same when compared to reaction mixers prepared just before the incubation step (Figure 3). Prepared and kept in this way, only 1-2 ul of DNA per sample are needed to perform the assay.

## Conclusions

The developed LAMP assay for *Cronartium ribicola* looks very promising as a specific, sensitive, and quick method to detect this pathogen in field samples. With prepared pre-mixed reaction mix tubes and pre-loaded Chelex 100 tubes, it takes a little more than one hour to get results. However, more samples need to be characterized by both Sanger sequencing of the ITS region and by LAMP to fully validate this assay.

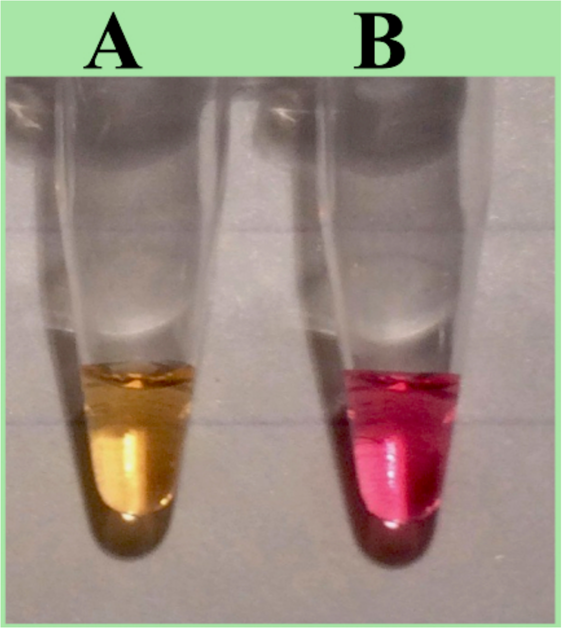

***Figure 3:*** *LAMP assay with pre-mixed reagents and stored at -20 C. A: 1 ng* C. ribicola *DNA; B: negative control.*

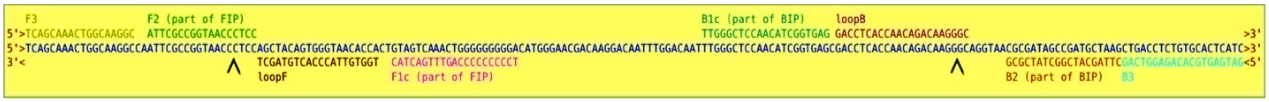

***Figure 4:*** Cronartium ribicola *genomic region (blue) showing the location of the different LAMP primers. Sequences above the genomic region are depicted in the 5' > 3' sense; those below are showed in the opposite sense (3' > 5'). F1c and F2 were connected by four "T" nucleotides to form FIP primer. B1c and B2 were connected by four "T" nucleotides also to form BIP primer. Arrow heads point to the limits of the region reported by Bergeron et al. (2019).*

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NATIONAL PARK
ESTABLISHED IN 1915

# COLONIZATION OF *POPULUS TRICHOCARPA* STEMS BY DIFFERENT *SPHAERULINA MUSIVA* ISOLATES

**Susanna Keriö[1], Kathy Cook[1], Kelsey L. Søndreli[1], and Jared M. LeBoldus[1,2]**

Pathogens of poplars have global negative impacts on biomass production in poplar plantations. In North America, the ascomycete *Sphaerulina musiva* causes Septoria leaf spot and stem canker of several poplar species and their hybrids. Despite the devastation caused by *S. musiva* in poplar plantations, the knowledge of host-pathogen interaction at the cellular level in this pathosystem is incomplete. For instance, knowledge of the nutrient acquisition strategy of *S. musiva* could open novel avenues of exploration in the host-microbe interaction in *Populus*. We studied the interaction of five *P. trichocarpa* genotypes (BESC-235, BESC-400, CMBF-28-1, GW-9823, GW-11026, phytozome.jgi.doe.gov) and twelve *S. musiva* isolates (11-19C, 11-20A, ARK05, LPH, LPH02, MN2, MN14, MN20, TAIGA125, TAIGA159, TAIGA168, TAIGA259) (Tabima et al. 2020) at the host-pathogen interface. The five *P. trichocarpa* genotypes were grown in a greenhouse and spray-inoculated (LeBoldus et al. 2010) with the 12 *S. musiva* isolates. Spray-inoculated stem samples with visible cankers were collected three weeks post inoculation for histological analysis. The samples were fixed in formalin-acetic acid-alcohol (10%-5%-50%), dehydrated in an alcohol series, and embedded in plastic (Technovit 7100 GMA Embedding kit). Sections 5-10 µm thick were stained with toluidine blue (TBO, 0.5% in 0.2 M citrate buffer, pH 4.2), and mounted in acrylic resin mountant (Poly-Mount, Polysciences). We observed extensive host colonization in the samples inoculated with the more aggressive isolates compared to the less aggressive isolates. In response to pathogen infection, tylose formation in xylem vessels and deposition of phenolic compounds in phloem was induced. The hyphal colonization was primarily intercellular as observed earlier (Weiland & Stanosz 2007), and no consistent evidence of direct cell penetration or intracellular feeding structures in living cells was found. The hyphae were observed to colonize the intercellular spaces next to living cells, with indication of pathogen-induced changes in cell viability. We are conducting further experiments to explore the mode of host cell death induced by *S. musiva*.

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# DEVELOPMENT OF TOOLS FOR DETECTING, MONITORING, AND MANAGING BROWN ROOT ROT (CAUSED BY *PHELLINUS NOXIUS*) IN THE PACIFIC ISLANDS

**Mee-Sook Kim[1], Andrea R. Garfinkel[2], Phil Cannon[3], Ned B. Klopfenstein[4], and Jane E. Stewart[2]**

## Introduction

The brown root rot pathogen (*Phellinus noxius*) is causing a destructive, mortality-causing disease on diverse tropical trees that are integral to forest ecosystems in the Pacific Islands (e.g., Ann et al. 2002, Brooks 2002, Sahashi et al. 2015, Figure 1). Our previous work indicates that (1) this invasive fungal pathogen is more prevalent and aggressive than previously reported across the Pacific Islands (e.g., Northern Mariana Islands, Federated States of Micronesia, Palau, American Samoa); and (2) multiple genetic groups of this fungal pathogen are present, which indicates that the pathogen can differ across eastern Asia, Australia, and Pacific Islands. To date, *P. noxius* has not been reported in some U.S. states/territories (e.g., Hawaii, Florida, Puerto Rico) where it represents a major invasive threat based on our bioclimatic models (e.g., Figure 2). Currently, human-associated spread of the pathogen into uninfested areas is a major concern exemplified by possible movement via infected plants, shipping containers, and/or soil associated with equipment/vehicles in the Pacific Islands and other U.S. states/territories. Currently, little or no information is available on (1) identification/detection of *P. noxius*, (2) brown root rot disease incidence/severity, pathways of spread, and (3) disease management in regions impacted or threatened by this invasive forest pathogen. Here, we present preliminary approaches to develop molecular probes for detecting distinct genetic groups within *P. noxius*. In addition, we are working to develop DNA-based tools for assessing the effectiveness of management methods for brown root rot disease, which are essential but critically lacking. The DNA-based tools developed from this project will be used to detect and monitor the brown root rot pathogen, while also helping to determine effective treatments to minimize damage from the disease in vulnerable areas within the Pacific Islands. Here, we present preliminary approaches toward developing tools to detect and monitor the soil/rhizosphere inoculum density of invasive *P. noxius* over time and determine the effects of treatments (e.g., urea, lime, urea + lime, and control) for disease management. In addition, other soil microbiota will be also monitored in association with management treatments.

## Materials and Methods

### Planned experimental site and design:

The project site will be selected among *P. noxius* infection centers within the Pacific Islands (e.g., Guam, Rota, Saipan, or Pohnpei). Approximately 30 infected trees will be selected to serve as center for each treatment plot of ca. 12 m (40 ft) diameter (0.0113 ha). After removing infected trees, including excavating roots and root residues (> 0.6 cm diameter), and collecting pre-treatment samples, one of five treatments will be applied to each plot. The five treatments follow:

1. Urea at a rate of 336 kg/ha (300 lbs/acre)
2. Urea at a rate of 112 kg/ha (100 lbs/acre)

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3. Urea at a rate of 336 kg/ha (300 lbs/acre) plus lime to pH 7.0
4. Urea at a rate of 112 kg/ha (100 lbs/acre) plus lime to pH 7.0
5: Control (untreated)

Our experimental design will be a complete randomized block design with six replications (30 plots = treatment/plot x 5 treatments x 6 replications). Soil probes to record temperature and moisture will be installed at each plot, and soil cores will be taken to determine soil properties.

***Figure 1:*** Phellinus noxius *infection - dried mycelial sheath/crust (Photo: by Fred Brooks).*

### Soil extractions, DNA extractions, metagenomic analyses, and tree plantings:

To determine changes in *P. noxium* density over time, soils will be collected before (baseline density of *P. noxius*) and after treatment applications in each plot to quantify *P. noxius* inoculum (e.g., every 3-months for 24 months) as well as to identify soil microbes (fungi and bacteria) associated with potential biological control of brown root rot disease. Metagenomic (metabarcoding) tools will be used for comparing forest soil microbial communities to (1) determine differences among soil treatments, (2) assess how differences relate to the soil properties (chemical and physical), and (3) examine how communities compare across space and time. At 6-month post-treatment, three breadfruit propagules will be planted in each plot to allow monitoring of future *in vivo* assessments of treatment effects on tree infection over the long-term.

### qPCR/PCR:

In addition to rDNA-based, DNA barcoding, we plan to develop a DNA probe that distinguishes *P. noxius* from other soil microbes. This particular probe will be used for real-time PCR, which provides detection and quantifies relative pathogen amounts based on the DNA amplification rates. DNA extracted from control soils will also provide a basis for quantifying relative pathogen levels. Real-time PCR will be conducted on each soil sample to determine the relative changes in *P. noxius* levels associated with each soil treatment.

### Soil analyses:

Soil temperature and moisture will be recorded over time. Soil samples will also be tested for pH and stored for measuring other soil properties, such as organic matter, bulk density, etc. Soil information will allow comparisons with soil levels of *P. noxius* and other microbes.

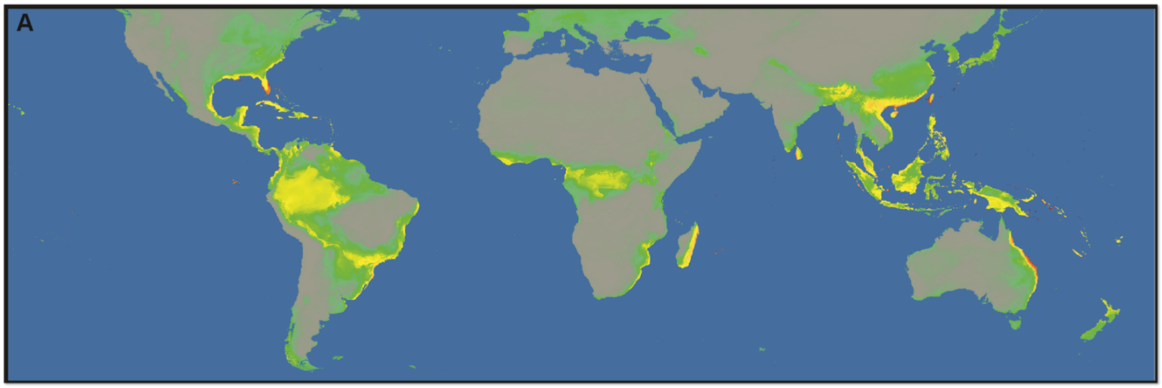

***Figure 2:*** *Maxent bioclimatic predictions of potential distribution (suitable climate space) for* Phellinus noxius *based on all DNA confirmed records of occurrence. Different colors represent probability of risk based on suitable climate space for* P. noxius *(Modified from Stewart et al., in revision). Light green represents predicted suitable climate space, with green, yellow, orange, and red indicating increased suitability, respectively.*

### Benefits

The tools developed from this project will be used to determine appropriate soil treatments and associated management methods for minimizing damage from brown root rot disease in vulnerable areas within the Pacific Islands. The scientific information provided by this project is critical to continuing development of sustainable management methods (e.g., fostering natural biological control and/or application of other treatments) for brown root rot disease.

## Acknowledgements

This project is supported by USDA – Forest Service, State and Private Forestry, Forest Health Protection – Special Technology Development Program, Pacific Northwest Research Station – Threat Characterization and Management Program, and Rocky Mountain Research Station – Forest Woodland Ecosystems Program.

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# PHYLOGENETICS AND HOST DISTRIBUTION OF *ARMILLARIA* IN RIPARIAN FORESTS OF THE NORTHERN GREAT PLAINS

**Shawn B. McMurtrey[1], Brandon C. Alveshere[2], Patrick I. Bennett[1,3], Mee-Sook Kim[4], John W. Hanna[5], Ned B. Klopfenstein[5], James T. Blodgett[6], and Jared M. LeBoldus[1, 7]**

## Abstract

Root disease pathogens, including *Armillaria* (Fr.) Staude*,* are a leading cause of growth loss and mortality of trees in forest ecosystems of North America (Lockman & Kearns 2016). This panglobal fungus can cause significant reductions in tree growth that can lead to mortality. *Armillaria* spp. have a wide host range, and they also play a vital ecological role in the carbon cycling process via wood decomposition (Raabe 1962, Baumgartner et al. 2011, Heinzelmann et al. 2019). As a native root-disease fungus, *Armillaria* spp. are among the oldest and largest known living organisms on Earth (Ferguson et al. 2003). Armillaria root disease has been found in every region of the United States. Currently, 11 species of *Armillaria*/*Desarmillaria* are known to occur in North America (Kim et al. 2006, Klopfenstein et al. 2017, Elías-Román et al. 2018), each with different ecological roles from pathogenic to saprophytic. Very little work has been done identifying and characterizing the distribution of *Armillaria* spp. in the northern Great Plains region, although *A. gallica* Marxm. & Romagn was previously identified in association with diverse hardwood species in the Niobrara Valley Preserve in Nebraska (Kim & Klopfenstein 2011).

Phylogenetic analyses provide a better understanding of taxonomic relationships among *Armillaria* species, which facilitates mapping spatial distributions and host associations for insights into *Armillaria* ecology. The objectives of this study were to: 1) provide baseline information on *Armillaria* species that are present in the northern Great Plains region; and 2) determine geographic distribution of host trees that are associated with the *Armillaria* species. A broad goal of this study is to provide important missing data that might assist with updating root disease risk maps. Isolates were collected from 78 out of 101 sites surveyed. Host species was recorded, and each tree was inspected for signs/symptoms of root disease (killing of the cambium in a live tree) and butt rot (degrading inner wood in a live tree) (Williams et al. 1986). A total of 57 *Armillaria* isolates were sequenced from 12 different host tree species from sites in North Dakota, South Dakota, and Nebraska. Identification of *Armillaria* isolates was accomplished by comparing nucleotide sequences from the translation elongation factor 1-alpha (*tef1*) gene. All 57 isolates had a >98% general consensus match to *A. gallica*. The locations of *A. gallica* were then mapped (Figure 1). A phylogenetic tree was constructed using maximum-likelihood (ML) algorithms (Figure 2). Statistical support for the branch topology was calculated from 1,000 bootstrap replicate trees. *Armillaria gallica* has a wide host range and geographic distribution, and it is much more widespread in the northern Great Plains region than previously recognized.

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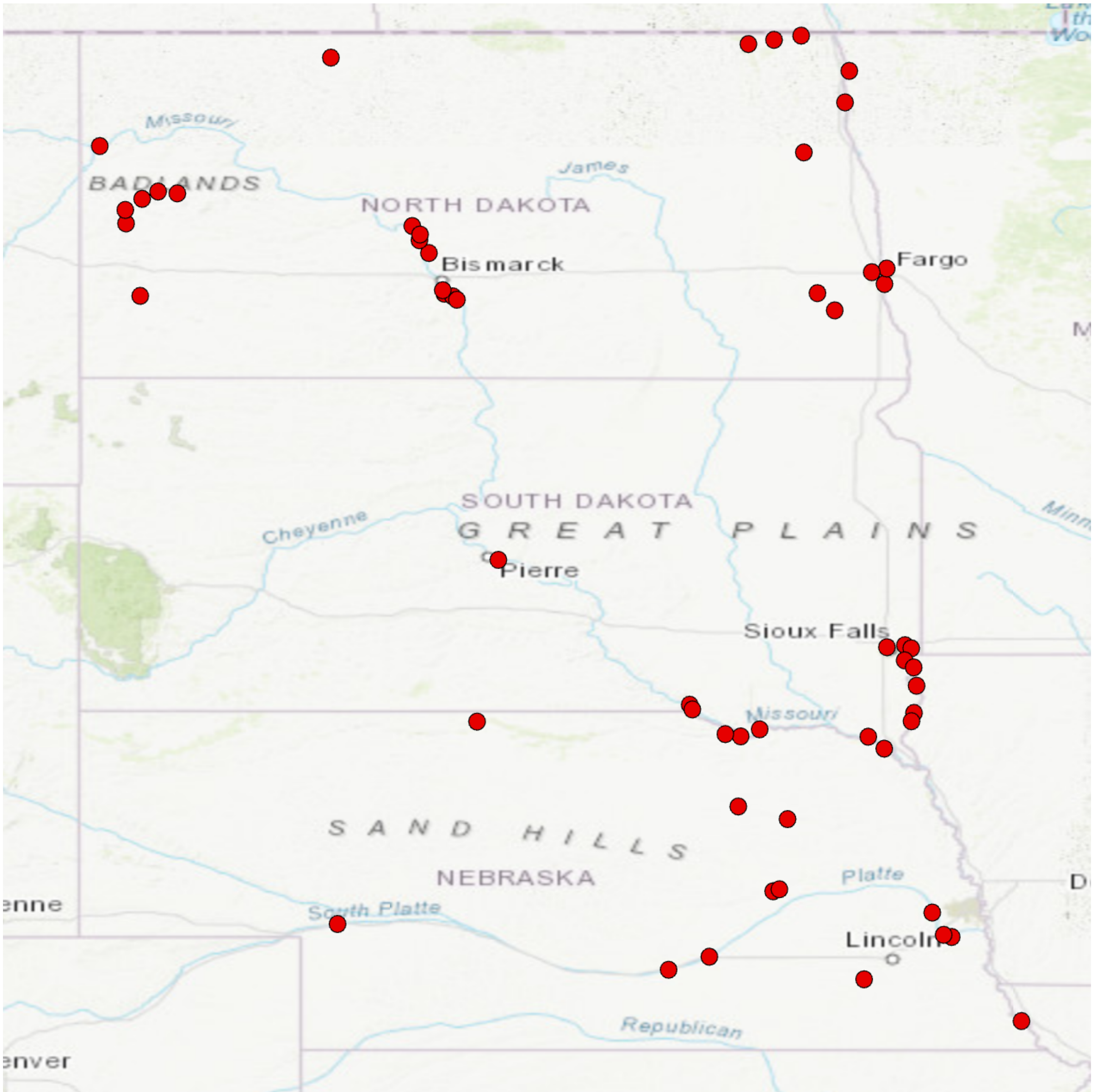

***Figure 1:*** *This map depicts the 57 locations where* Armillaria gallica *was found. Each isolate that was sequenced had pairwise identity matches with* A. gallica *at a > 98% match using translation elongation factor 1-alpha gene comparison.*

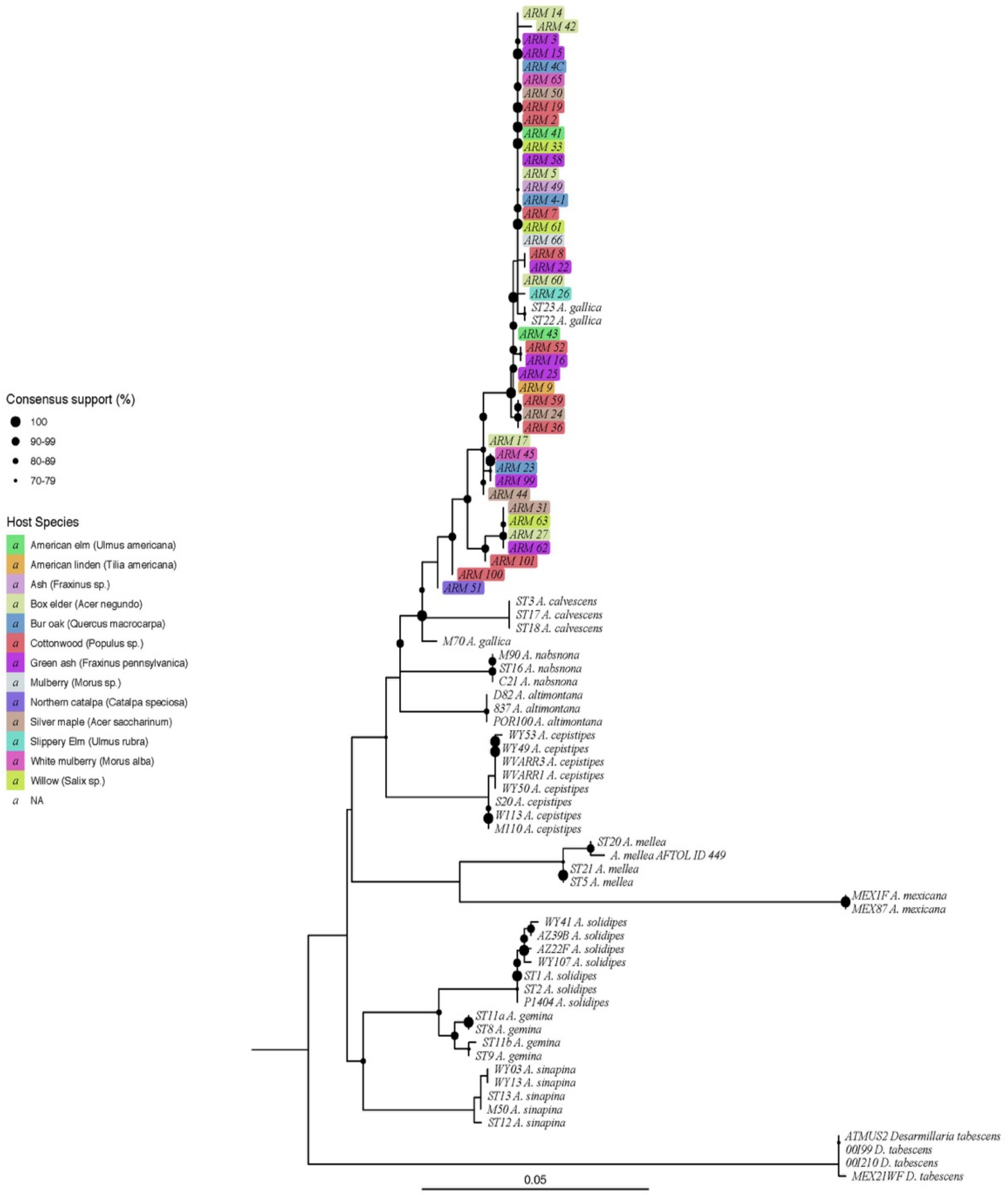

***Figure 2:*** *Phylogenetic trees were constructed using maximum-likelihood (ML) algorithms, and statistical support for the branch topology was calculated from 1,000 bootstrap replicate trees. Colors represent host species shown in the legend. The sizes of the circles at nodes represent bootstrap support. Only values > 70% are shown. This phylogenetic tree shows 42 of the 57 isolates that were sequenced.*

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# EVALUATING COMBINATION TREATMENTS (PRUNING AND THINNING) TO REDUCE WESTERN DWARF MISTLETOE IN YOUNG PONDEROSA PINE PLANTATIONS IN CENTRAL OREGON

**Brent Oblinger[1] and Will Brendecke[2]**

## Introduction

Various management strategies are utilized in the western U.S. to reduce dwarf mistletoe in ponderosa pine to maintain healthy, vigorous plantations. In pure pine plantations, thinning, overstory removal/stand replacement, species manipulation/planting nonhost buffers, and prescribed burning are common tools for managing western dwarf mistletoe (*Arceuthobium campylopodum*). Pruning mistletoe brooms in conifers has been a management strategy in recreation sites (Lightle & Hawksworth 1973, Scharpf et al. 1988, Maffei et al. 2016), but less pruning has been implemented at broader scales in managed forest stands. However, limited operational mistletoe pruning has occurred in some ponderosa pine stands on the Deschutes National Forest throughout the past (e.g., Knutson 1975, Maffei 1999). Some of the earliest descriptions of pruning to reduce dwarf mistletoe were proposed by Perry (1922) and Weir (1923). Combination treatments are evaluated that include pathological pruning along with thinning (via felling or girdling) severely infected pines on the Deschutes National Forest. All treatments were implemented by Youth Conservation Crews (YCC's) in over 20 plantations on the Sisters Ranger District between 2015 and 2018.

## Methods

Younger ponderosa pine plantations (< 50 yr old) were identified using stand-based inventory data in ArcMap (ver. 10.2, ESRI, Redlands, CA) within Project Planning Areas known to have dwarf mistletoe management concerns (Figure 1) and where National Environmental Policy Act documentation was completed. Plantations were prioritized for treatments based on the following field observations: incidence of dwarf mistletoe (low-to-moderate prioritized), tree heights for prunability, and road access. Mechanized and non-mechanized pole saws were used for pruning, and chainsaws were used for tree girdling and felling. Trees were to be pruned with a Dwarf Mistletoe Severity Rating (DMR; Hawksworth 1977) of 1-4 if at least 25% of their existing live crown could be retained. Two whorls above visible branch infections were to be pruned whenever possible to reduce undetectable, latent infections. Trees with a DMR of 5-6 were to be girdled or felled by hand. Slash was piled away from the base of trees (Figure 2).

Post-treatment surveys were conducted in five plantations with fixed-radius plots randomly distributed (2/ha) to evaluate the incidence and severity of western dwarf mistletoe. The 2017 Milli Fire also burned following treatments in one plantation surveyed. Post-treatment surveys occurred within one month to 18 months post-treatment. Both pre- and post-treatment surveys were conducted in one of the five plantations. Hawksworth's 6-Class Dwarf Mistletoe Rating System (1977) was applied for rating infection severity. A Mann-Whitney test ($\alpha = 0.05$) was used to test whether the DMR was different in plots with dwarf mistletoe before vs. after treatment. Heights to pruned branches were measured to estimate the upper height limit of prunable trees with pole saws that were extendable to 4.9 m. Compacted live crown ratios (LCRs) were measured to evaluate the amount of live crown retained after pruning.

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***Figure 1:*** *Western dwarf mistletoe on ponderosa pine in a plantation being considered for treatment.*

***Figure 2:*** *Examples of post-treatment stand conditions where pathological pruning occurred.*

***Table 1:*** *Stand summary statistics from post-treatment surveys in five plantations.*

| Plantation ID | Treatments Implemented | Mean DBH of Trees (cm) | Mean Height of Trees (m) | Mean Incidence of Dwarf Mistletoe (%) | Stand DMR | Mean Height of Highest Branch Pruned (m) | Mean Live Crown Ratio of Pruned Trees (%) |
|---|---|---|---|---|---|---|---|
| 0-17 | Pruning & thinning | 29.4 | 14.3 | 5.8 | 0.08 | 4.3 | 63 |
| 3-17 | Pruning & thinning | 29.7 | 14.5 | 16.9 | 0.31 | 4.5 | 57 |
| 4-18P | Pruning & thinning | 25.8 | 11.9 | 2.2 | 0.02 | 3.5 | 64 |
| D5-4 | Pruning & thinning | 24.8 | 11.1 | 6.6 | 0.07 | 3.8 | 60 |
| SAFR24 | Pruning, thinning & wildfire | 23.4 | 10.4 | 5.4 | 0.07 | 3.4 | – |

## Results and Discussion

In plantations surveyed, the mean diameter at breast height (DBH; 1.37 m) and height of trees ranged 23.4 - 29.7 cm and 10.4 - 14.5 m, respectively (Table 1). Low incidence (means 2.2 - 16.9%) and severity (mean stand DMRs 0.02 - 0.31) of mistletoe were observed post-treatment, but a few trees were left untreated, or not all infected branches were pruned in a few trees. A significantly lower ($p = 0.028$) DMR was observed in treated plots post-treatment in the plantation where pre- and post-treatment surveys were conducted (Figure 3). Mean heights to the highest branch pruned were 3.4 - 4.5 m and the highest prunable branches were ~6.7 m. Sufficient LCRs remained post-pruning with mean LCRs ranging 57 - 64% in pruned trees. The rate for crews of 5-6 was ~$400 per day, and 0.81 - 2.0 ha per day were treated. If specifications were not met, YCC's returned to some plantations to further reduce incidence and severity of mistletoe.

Although treatments did not completely sanitize plantations of mistletoe, stand DMRs were very low post-treatment and inoculum loads were reduced. Thinning was primarily completed along the edge of plantations with adjacent, overstory inoculum sources. Edge management in plantations and adjacent stands should be emphasized to enhance treatment effectiveness in plantations with low-to-moderate incidence of dwarf mistletoe. It is unknown how long combination treatments can reduce stand DMRs before potentially reaching pre-treatment DMRs again, and long-term plots could be established to evaluate treatment effects over time.

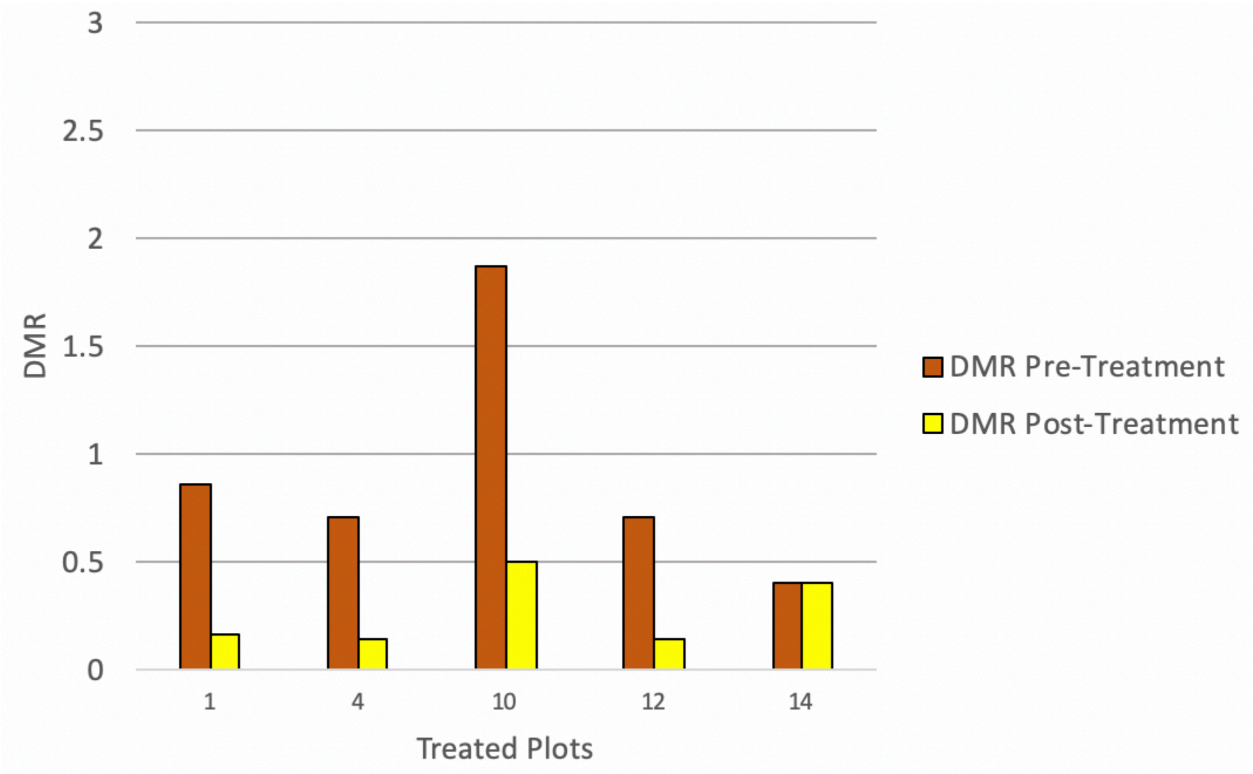

***Figure 3:*** *Mean DMRs of treated plots in Plantation D5-4.*

Prescribed underburning before or after pruning and thinning, or pile burning post-treatment, could be considered to reduce dwarf mistletoe (Conklin & Geils 2008). Even after a wildfire burned in one plantation surveyed post-treatment, low levels of mistletoe were still observed. Crown lifts along roads increase canopy base height reducing ladder fuels and can enhance fuel breaks. Pruning branches in the lower bole to decrease knots in wood could potentially add value to future logs as well. Firewood sales could be considered where thinning occurs along roads to reduce surface fuels from slash created during treatments. The risk of bark beetle activity (e.g., *Ips* spp.) following treatments should be evaluated where larger-diameter trees are thinned and slash is on-site, but bark beetles did not appear to be a concern here post-treatment. The minimum amount of live crown to retain after pruning could be investigated further (Knutson 1975). The use of airborne lidar data to identify plantations with prunable trees in a certain height range could also be evaluated during planning efforts. Under close supervision of YCC's or other contractors, combination treatments (pruning and thinning) can be another tool for managing western dwarf mistletoe in young ponderosa pine plantations when funding is available.

## Acknowledgements

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# FORTY YEARS OF *HETEROBASIDION OCCIDENTALE* ROOT DISEASE IMPACTS IN CALIFORNIA FIR FORESTS

**Adrian Poloni[1] and Richard Cobb[1]**

Tree mortality from the interaction of drought, bark beetles, and root disease continues to challenge forest management across the Sierra Nevada Mountain Range of California. Here we report over 40 years of continuous monitoring data in twenty-three *Heterobasidion occidentale* root disease centers in mid-elevation (1000-2000m) mixed evergreen forests. This data was leveraged to estimate rates of root disease center expansion and the longevity of individual disease centers. Total area affected by root disease ranged from 138-606$m^2$ and rate of spread ranged from .56-7$m^2$/year, which are considerably smaller and slower compared to *H. irregulare* (Pine type). Further work is aimed at understanding if root disease center dynamics drive *Scolytus ventralis* bark beetle outbreaks and thereby play a role in larger fir mortality. Knowledge of root disease center dynamics and their association with bark beetle populations can enhance predictions of bark beetle outbreaks and further inform strategies to mitigate tree mortality in fir forests of the Sierra Nevada Mountain Range of California.

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Jim
Blodgett

# USING ROADSIDE SURVEYS TO MAP DISTRIBUTION AND INCIDENCE OF SOUTHWESTERN DWARF MISTLETOE ON PONDEROSA PINE IN NEW MEXICO NATIONAL FORESTS

**Gregory J. Reynolds**[1]

## Introduction

*Arceuthobium vaginatum* subsp. *cryptopodum* (Engelm.) Hawksworth & Wiens causes southwestern dwarf mistletoe (SWDM) on ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) in the southwestern US and is among the most damaging pathogens affecting this host in New Mexico. Dwarf mistletoes reduce growth rate, increase susceptibility to fire, and alter branch architecture by inducing formation of witches' brooms. Although native to the state, historic management has led to overstocking that has facilitated increased spread and severity. Because dwarf mistletoe cannot be detected using aerial surveys, as with damage associated with insects, a different survey method is needed. Roadside surveys would be one potential tool for conducting these assessments of dwarf mistletoe incidence. Various types of roadside surveys have been utilized in other regions and shown to be accurate for assessing relative incidence of dwarf mistletoe diseases in multiple hosts (Mathiasen et al. 1996, Merrill & Hawksworth 1985). Roadside surveys have previously been conducted in New Mexico national forests at roughly 30 year intervals beginning in 1960 (Andrews & Daniels) and subsequently in the late 1980's (Beatty et al. 1987, Maffei et al. 1987a., Maffei et al. 1987b) to rapidly assess relative SWDM incidence across large areas. These previous surveys did not include a spatial component, so although the reports provide summaries of SWDM incidence and damage associated with the parasite, there is no indication of the location of infections. Beginning in 2017, roadside surveys were conducted along roadways in areas with extensive ponderosa pine to systematically map the distribution and incidence of SWDM in national forests of New Mexico. Results are being provided to land managers in the US Forest Service to assist with planning efforts.

## Methods

During 2017 and 2018, roadways in the five New Mexico national forests were traversed at 16-24 km/h with an observer noting relative incidence of SWDM along 0.16 km road increments to a depth of one chain (20m) into the forest. Relative incidence of SWDM was noted for each road increment on a scale of 0-3, with 0: no SWDM observed, 1: < 33% incidence, 2: 33-66% incidence, and 3: > 66% incidence. A relative host size class was also assigned, and the presence of thinning treatments and/or burned areas was noted for each increment. Data were entered into ArcGIS using Survey123, and maps were generated showing the relative incidence of SWDM along roadways.

To validate the roadside survey method, 54 variable-radius prism plots (BAF 10) were established in a subset of areas representing all relative incidence levels. Basal area ($m^2$/ha) and dwarf mistletoe ratings (DMR, Hawksworth 1977) were calculated for each plot and averaged for each incidence rating.

## Results and Discussion

The three levels of relative SWDM incidence identified by this study roughly correlate with management guidelines for the southwest region (Conklin & Fairweather 2010). Briefly, lightly infected areas (< 20% incidence ≈ roadside survey level 1) may be treated similarly to uninfected stands, using group selection or

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individual tree selection to maintain an uneven aged structure and eliminate small pockets of disease. Moderately infected areas (20-80% incidence ≈ roadside survey level 2) may require more intensive, even-aged treatments such as shelterwood or seed tree cuts. Leave trees should be the healthiest and most lightly infected host trees available and should have a maximum DMR of 2 or 3. Prescriptions should also be flexible, and uninfected or more lightly infected areas within an overall moderately infected stand may be maintained in an uneven-age structure. Finally, severely infected stands (> 80% incidence ≈ roadside survey level 3) should generally be replaced if possible or deferred from management, as limited funding is often better spent managing healthier stands.

In 2017 and 2018, 380.3 km of roadway were surveyed across the five New Mexico national forests, representing 2,363 total 0.16 km road increments (Figure 1). Overall, no SWDM was observed on 1,568 of these increments (66.4%). Light incidence where < 33% of stems were infected was identified on 443 increments (18.7%). Moderate incidence where 33-66% of stems were infected was identified on 212 increments (9.0%). Severe incidence where > 66% of stems were infected was identified on 140 increments (5.9%). Previous work identified SWDM at some level of incidence across 36% of all plots surveyed in Arizona and New Mexico, indicating that the area covered by infestations across the region has remained somewhat consistent for over a half century (Andrews & Daniels 1960). Maps generated from this work are being provided to forest managers for planning. An example map for one extensively surveyed area is provided for the western Zuni Mountains of the Mount Taylor Ranger District, Cibola National Forest (Figure 2). Data from this area are already being used to plan work associated with a large Collaborative Forest Landscape Restoration Program project.

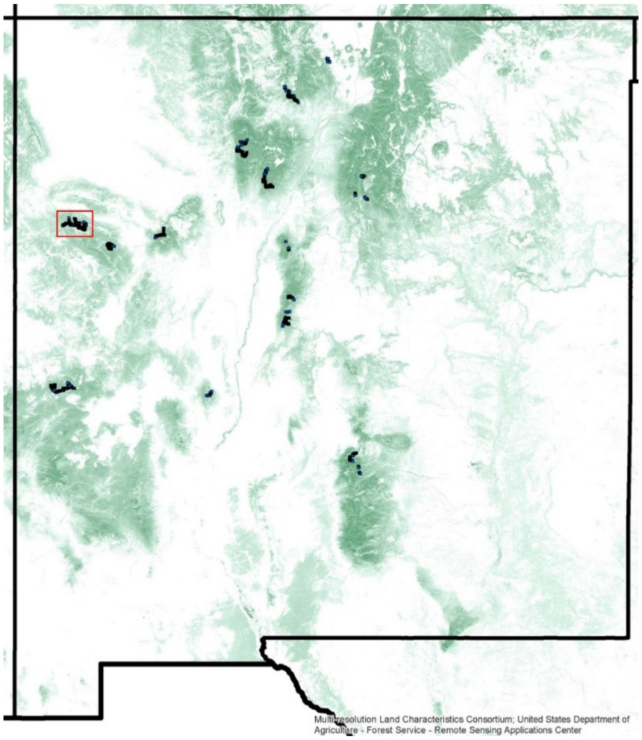
Multiresolution Land Characteristics Consortium; United States Department of Agriculture - Forest Service - Remote Sensing Applications Center

***Figure 1:** Location of 2017-2018 southwestern dwarf mistletoe roadside surveys in New Mexico (black points); the highlighted cluster of points represents the western Zuni Mountains of Cibola National Forest and is shown in detail in Figure 2.*

Based on past surveys, SWDM incidence increased in some areas (e.g. 59% with some level of infection in 1960 compared to 69% in 2017 for the Santa Fe National Forest) and declined in others (e.g. 79% with some level of infection in 1987 compared to 74% in 2017 for the Mount Taylor Ranger District, Cibola National Forest). Incidence may have increased in some cases due to natural intensification in unmanaged areas as well as spread into previously healthy stands from neighboring infection centers. Reduced incidence is likely due to wildfire destroying heavily infected stands as well as management operations. It should be noted that because the previous surveys did not have a geospatial component, it is impossible to directly compare the recent surveys with those from 1960 and 1987. These recent surveys should be treated as new baseline data that can be more accurately remeasured in the future.

Within variable radius prism plots, no dwarf mistletoe was found in areas rated 0 during roadside surveys (DMR = 0). Areas rated 1 during roadside surveys had an average incidence of 11% within plots (DMR = 0.2), which was within the predicted range of 1-32% incidence. Areas rated 2 during roadside surveys had an average incidence of 49% within plots (DMR = 1.2), which was within the predicted range of 33-66%. Areas rated 3 during roadside surveys had an average incidence of 71% within plots (DMR = 2.3), which was within the predicted range of 67-100%. These plots confirmed the accuracy of the roadside survey method as a reliable and rapid measure of SWDM incidence. There were no significant differences in average basal area between the different roadside survey incidence levels (21.1-23.7 $m^2$/ha).

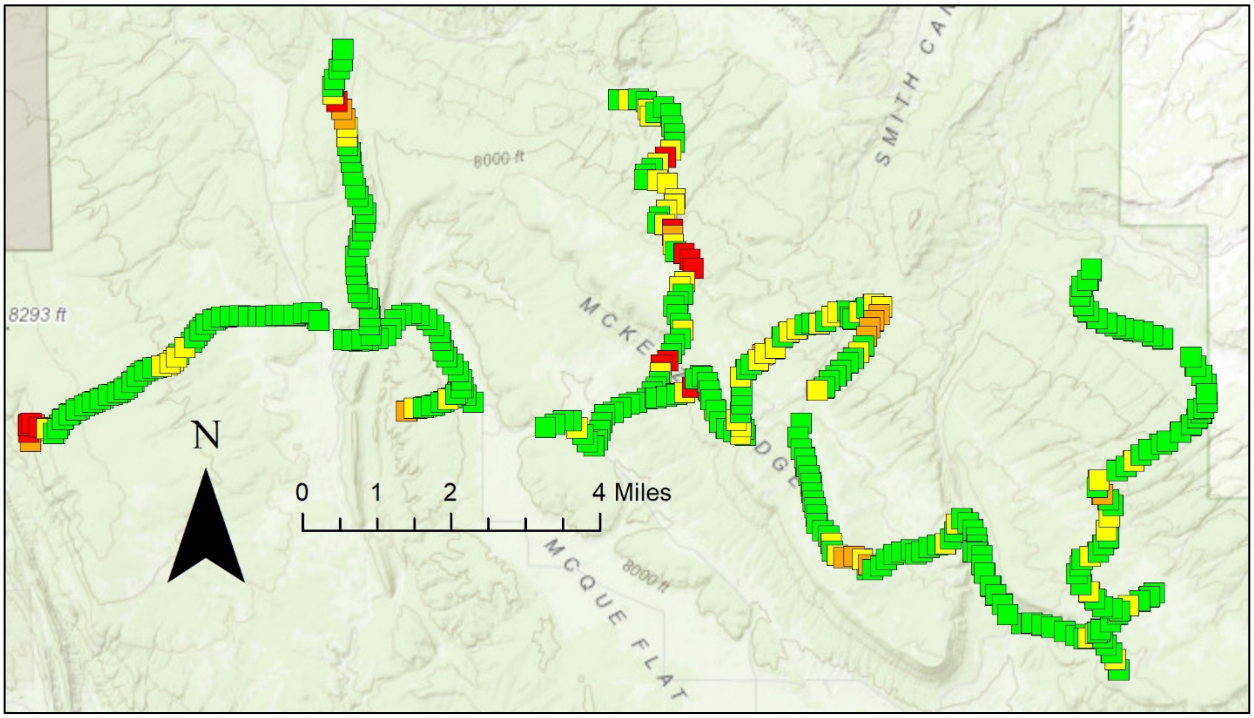

***Figure 2:*** *Southwestern dwarf mistletoe incidence from roadside surveys in the western Zuni Mountains (Cibola National Forest); green indicates no mistletoe observed, yellow indicates < 33% incidence, orange indicates 33-66% incidence, and red indicates > 66% incidence.*

Southwestern dwarf mistletoe was an ideal parasite to map using this roadside survey method because of its large, bright orange shoots and consistent production of distinctive witches' brooms in infected trees. The Survey123 collection tool used in this study may also be used to map incidence of other dwarf mistletoe species in New Mexico. Mapping Douglas-fir dwarf mistletoe (*A. douglasii* Engelm.) occurred in some areas where it was noted. Although shoots of this species are small and difficult to identify during roadside surveys, consistent and obvious witches' brooms do make identification of infections from the roadway possible. This species was nevertheless not as amenable to systematic mapping as ponderosa pine, however, because of disjointed host populations. This would not be the case for two-needle pinyon pine (*P. edulis* Engelm.), as extensive areas of this host occur in the state that are well covered by road systems, but trees infected by pinyon dwarf mistletoe (*A. divaricatum* Engelm.) do not consistently produce witches' brooms, and shoots of the parasite are usually green or brown in color and not as easily identified during roadside surveys as those of SWDM. Roadside surveys have previously been used to map lodgepole pine dwarf mistletoe (*A. americanum* Nutt. ex Engelm.) in Idaho (Mathiasen et al. 1996), and the tool would likely be useful for a number of other dwarf mistletoe species and hosts in other regions of the U.S. Forest Service.

## Acknowledgements

The author thanks Tom Coleman, former New Mexico Zone Leader, for proposing and initiating this work; student technicians Josiah Pettit, Jesse Nieto, and Chris Peralta for assistance with data collection; and Andy Graves, current New Mexico Zone Leader, for providing a helpful review of this manuscript.

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# MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF ROOT DISEASES IN THE WESTERN USA

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Doug J. Westlind[3], Josh J. Bronson[9], Amy C. Ramsey[10], Sara M. Ashiglar[11], Melissa J. Fischer[12],
Bill Woodruff[13], Greg J. Reynolds[14], and Ned B. Klopfenstein[1]**

## Introduction

Root diseases cause extensive damage to forests in the western USA (Blodgett et al. 2015). However, despite their abundance and economic impact (Lockman et al. 2016), we know very little about the identity and distribution of some pathogens responsible for these diseases. Our project addresses this knowledge gap by characterizing root pathogens in forests of the western USA (Kim et al. 2006) and has the potential to result in updated distribution maps, bioclimatic models, as well as first reports.

## Materials and Methods

Forest Health Specialists in the western USA were asked to collect samples of diverse root diseases. In 2018, a total of 204 root disease samples were collected from AK, CA, CO, NM, OR, SD UT, and WA by 16 professionals (Figure 1). Root disease samples were cultured to isolate the pathogen, and identified to species using DNA-based technology.

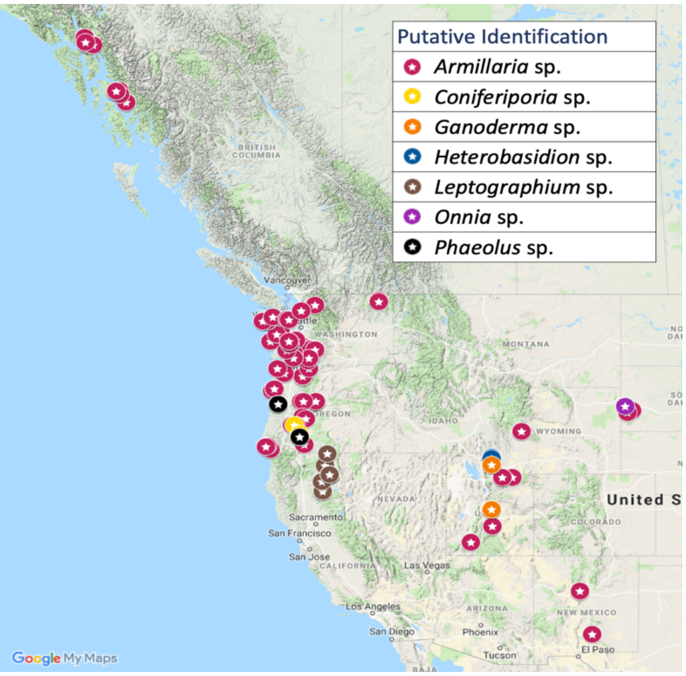

***Figure 1:*** *Map showing the collection locations for the 2018 root disease samples sent to the laboratory for culturing and DNA-based identification.*

## Results and Discussion

In total, 228 samples were obtained, yielding 116 successful cultures. Armillaria Root Disease (ARD) comprised the majority of samples (Figure 2), confirming that it is a major root disease in the western USA. DNA sequence-based identification, although in its initial stages, identified several ARD samples as *Armillaria solidipes* (= *A. ostoyae*), and other *Armillaria* spp. were confirmed. Black stain root disease (*Leptographium wageneri*) was the next most

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commonly sampled pathogen, but the methods employed to culture this pathogen were unsuccessful. Much less abundant in our samples are root diseasescaused by *Coniferiporia* (formerly *Phellinus*), *Heterobasidion*, *Phaeolus*, *Onnia*, and unknown pathogens. Overall, the success rate was highly variable for establishing pathogen isolates in culture. The pathogen, type of sample, storage conditions, and time stored before isolation likely influenced the success of pathogen isolations.

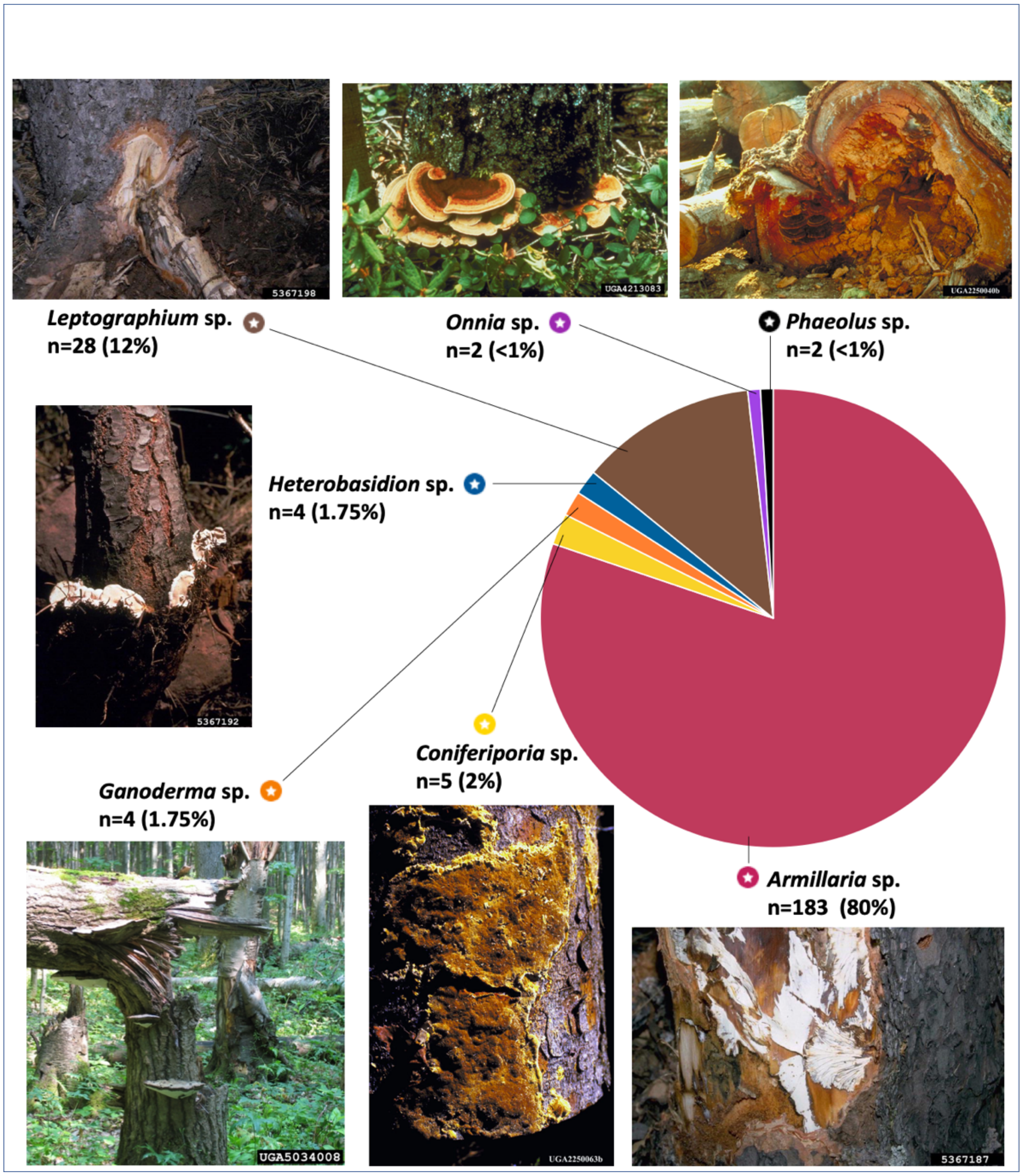

***Figure 2:*** *Pie chart showing the relative proportion of samples obtained that are presumably caused by the fungal genera pictured. Colors indicate different genera and coordinate with the map legend in Figure 1. Photos were obtained from Bugwood Image Database System and include:* Armillaria *spp. William Jacobi Colorado State University 5367187,* Heterobasidion annosum *Mike Schomaker Colorado State Forest Service 5367192,* Phaeolus schweinitzii *USFS Northern and Intermountain region UGA2250040b,* Onnia tomentosa *Minnesota Department of Natural Resources UGA4213083,* Ganoderma *Joseph Obrien USFS UGA5034008,* Coniferiporia weirii *USFS Northern and Intermountain region UGA2250063B, and* Leptographium wageneri*, William Jacobi, Colorado State University 5367198.*

## Conclusions

In the upcoming 2019 field season, adjustments such as expediting the time from sample collection to pathogen isolation and using alternate culturing techniques may impact results, especially for difficult-to-isolate pathogens such as *Leptographium*. Moving forward, ARD samples will be used to strengthen previous bioclimatic models of ARD in the western USA. Ultimately, this project will continue to expand upon our knowledge of the fungal species causing root diseases in the western USA and their distributions.

## Acknowledgements

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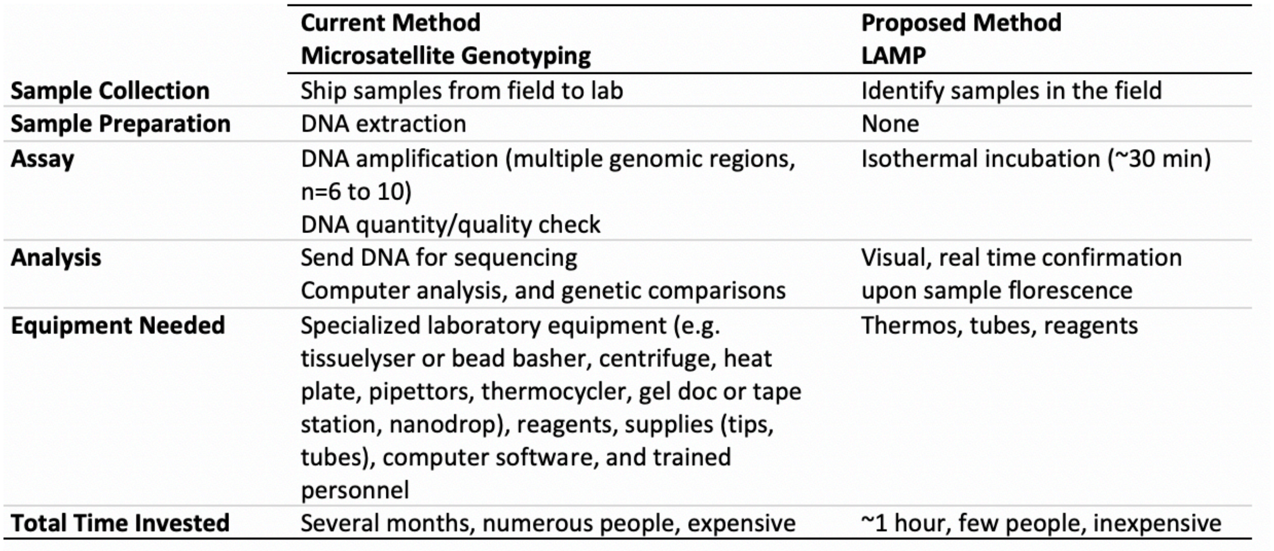
# THE NEED FOR RAPID MOLECULAR DIAGNOSTICS TO DISTINGUISH BIOTYPES OF THE MYRTLE RUST PATHOGEN (*AUSTROPUCCINIA PSIDII*)

**Rachael A. Sitz[1,2], Mee-Sook Kim[3], Phil G. Cannon[4], Acelino C. Alfenas[5], Morag Glen[6],**
**Alistair R. McTaggart[7], Ned B. Klopfenstein[1], and Jane E. Stewart[2]**

## Background

*Austropuccinia psidii* impacts numerous forest ecosystems world-wide by causing myrtle rust disease on many myrtaceous species, including guava *(Psidium guajava),* eucalypts *(Eucalyptus spp.)*, rose apple *(Syzygium jambos)*, and 'ohi'a *(Metrosideros polymorpha).* First reported in 1884 on guava in Brazil, the rust has since been detected in tropical and subtropical areas worldwide. At least three biotypes of *A. psidii* have currently been identified (Stewart et al. 2017), including eucalypt and guava biotypes in Brazil, and the globally distributed pandemic biotype which infects multiple hosts. However, despite known genetic variation in this species and distinct invasive threats, we currently lack rapid methods to quickly distinguish among different biotypes. This becomes especially problematic as biotypes of the pathogen continue to expand their geographic ranges. Although myrtle rust is established in Florida, California, Hawaii, Puerto Rico, Australia, and New Zealand, the introduction of new biotypes to these areas poses an additional threat, which could be amplified if hybridization were to occur (McTaggart et al. 2017, Loope & La Rosa 2008). Methods currently exist to confirm the presence of specific biotypes (Machado et al. 2015, Bini et al. 2018), but we need cost and time effective tools to quickly distinguish among biotypes (current and proposed methods are compared in Table 1). Here we propose a framework for developing rapid, user-friendly diagnostic assays for distinguishing among different *A. psidii* biotypes.

***Table 1:*** *Comparison of current and proposed methods for distinguishing* Austropuccinia psidii *biotypes.*



| | **Current Method**<br>**Microsatellite Genotyping** | **Proposed Method**<br>**LAMP** |
|---|---|---|
| **Sample Collection** | Ship samples from field to lab | Identify samples in the field |
| **Sample Preparation** | DNA extraction | None |
| **Assay** | DNA amplification (multiple genomic regions, n=6 to 10)<br>DNA quantity/quality check | Isothermal incubation (~30 min) |
| **Analysis** | Send DNA for sequencing<br>Computer analysis, and genetic comparisons | Visual, real time confirmation upon sample florescence |
| **Equipment Needed** | Specialized laboratory equipment (e.g. tissuelyser or bead basher, centrifuge, heat plate, pipettors, thermocycler, gel doc or tape station, nanodrop), reagents, supplies (tips, tubes), computer software, and trained personnel | Thermos, tubes, reagents |
| **Total Time Invested** | Several months, numerous people, expensive | ~1 hour, few people, inexpensive |

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## Objectives

This project aims to (1) develop rapid diagnostic assays, such as LAMP assays (Loop-Mediated Isothermal Application) to easily distinguish among the main myrtle rust biotypes; (2) further document the global distribution and host associations of each *A. psidii* biotype; and (3) provide stakeholder groups (such as quarantine officials and forest pathology researchers) with rapid diagnostic technology and training.

## Implications

This project will develop field assays to promptly detect different biotypes of *A. psidii.* The LAMP assay will greatly reduce the labor, time, and cost needed to biotype samples and improve our ability to respond to the invasive myrtle rust pathogen. Furthermore, it will guide regulatory and management practices that address potential invasive threats.

## Acknowledgements

We thank USFS STDP for funding and Jorge Ibarra Caballero for help in the experimental design.

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# COMPARING THE EPIDEMIOLOGY OF THE EU1 AND NA1 LINEAGES OF *PHYTOPHTHORA RAMORUM* IN OREGON FORESTS

**Kelsey L. Søndreli[1] and Jared M. LeBoldus[1,2]**

*Phytophthora ramorum,* the cause of Sudden Oak Death, is an invasive pathogen in California and Oregon. Forest infestations have all been the NA1 lineage until 2015 when the EU1 lineage of *P. ramorum* was found infecting a tanoak tree in southwestern Oregon. Tanoak (*Notholithocarpus densiflorus*) is the most susceptible species in Oregon and the main driver of this disease in the forest. Field experiments were conducted in 2017, 2018, and 2019 in order to evaluate the relative susceptibility of Douglas-fir*,* Sitka spruce*,* western hemlock*,* and western larch to the two lineages (NA1 and EU1) of *P. ramorum* under field conditions. In general, a larger proportion of trees and more tree species, based on re-isolation frequency, were infected at the EU1 site compared to the NA1 site. In 2018 and 2019, two rain bucket experiments were conducted to compare the inoculum produced at three NA1 infested sites and three EU1 infested sites. The results from these experiments indicate that the EU1 lineage of *P. ramorum* has the potential to infect more species and more trees within species than the NA1 lineage. The EU1 lineage may also have greater sporulation under field conditions, posing a greater threat to Oregon forests than the NA1 lineage.

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# MOLECULAR CHARACTERIZATION OF *FUSARIUM* SPECIES ASSOCIATED WITH DAMPING-OFF OF CONIFER SEEDLINGS IN TREE NURSERIES

**Jane E. Stewart[1], John T. Dobbs[1], Stephen Fraedrich[2], Gregory J. Reynolds[3], R. Kasten Dumroese[4], Ned B. Klopfenstein[4], and Mee-Sook Kim[5]**

## Abstract

*Fusarium* can cause significant damage within conifer nurseries across the United States. Several species of *Fusarium*, which are morphologically similar to *F. oxysporum*, are known to cause damping-off, including *F. commune*, *F. fujikuroi*, *F. proliferatrum*, and *F. solani*. Isolates of *Fusarium* spp. (ca. 431) were collected from Idaho, Oregon, Nevada, Washington, Nebraska, Michigan, North Carolina, South Carolina, and Georgia. Initial species identification was conducted based on colony morphology. Because *F. oxysporum* and *F. commune* have almost identical colony morphology with the exception that *F. commune* can sporadically produce polyphialides, DNA sequencing of translation elongation factor 1-α (*tef1*) gene was conducted to identify *Fusarium* isolates. To date, *tef1* sequences have been obtained for 345 of the 431 isolates of *Fusarium*. Using NCBI Blast, 76.7% of the isolates were identified as *F. oxysporum*, 8.5% as *F. commune*, 4.4% as *F. redolens*, 1.5% as *F. proliferatum*, 3.2% as *F. fujikuroi*, 1.2% as *F. solani,* and < 1% of *F. proliferatum*. Several isolates were also labeled *Fusarium* spp. Further work will also be discussed, such as the development of microsatellite markers for population studies of *F. commune* associated with different hosts and diverse geographic areas.

## Introduction

The genus *Fusarium* is ubiquitous in most container and bareroot nurseries on healthy and diseased conifer seedlings, in nursery soils, and on conifer seeds of several species, especially Douglas-fir (*Pseudotsuga menziesii*), western white pine (*Pinus monticola*), ponderosa pine (*P. ponderosa*), and loblolly pine (*P. taeda*) (James et al. 1990, Cram & Fraedrich 2009). Since the first report of Fusarium root rot in forest nurseries, the major pathogen was previously identified as *F. oxysporum* based on morphology (Bloomberg 1981). However, isolates of selected *Fusarium* spp. that had previously been characterized as pathogenic on Douglas-fir seedlings displayed a range of high, moderate, and low virulence (Stewart et al. 2006). Stewart et al. (2006) used DNA sequencing to determine that all the highly virulent isolates on Douglas-fir were *F. commune*, a recently named species (Skovgaard et al. 2003). Further, *F. proliferatum* was recently identified as a pathogen of sugar pine (*P. lambertiana*) in California (Stewart et al. 2016). DNA sequences from the mitochondrial small subunit (mtSSU) and translation elongation factor 1-alpha (*tef1*) regions are useful for distinguishing *Fusarium* species. Various *Fusarium* spp. have been reported as pathogens of conifer seedlings in tree nurseries. The objective of this study was to evaluate the occurrence and distribution of *Fusarium* species in conifer tree nurseries across the United States (Figure 1).

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## Methods

A total of 345 isolates of *Fusarium* spp. were collected in forest nurseries throughout the western, mid-western, and southern United States (Table 1). Isolates from each state were collected from one to five forest nurseries, as well as from diverse sources of hosts/substrates including (1) diseased or healthy seedlings of Douglas-fir, western larch (*Larix occidentalis*), western redcedar (*Thuja plicata*), yew (*Taxus* sp.), lodgepole pine (*P. contorta*), western hemlock (*Tsuga heterophylla*), western white pine, ponderosa pine, grand fir (*Abies grandis*), rabbitbrush (*Chrysothamnus* sp.), sagebrush (*Artemisia tridentata*), Austrian pine (*P. nigra*), loblolly pine, blue spruce (*Picea pungens*), bitterbrush (*Purshia tridentata*), and eastern redcedar (*Juniperus virginiana*), (2) containers of various conifer seedlings, and (3) soil/growing medium. All 345 isolates were characterized using mtSSU and/or *tef1*. Template DNA was derived from scrapings of actively growing mycelial cultures (3-5 days old) or using chelex, a quick DNA extraction method. The PCR products were sequenced at the University of Wisconsin – Biotechnology Center (Madison, WI, USA) or at Eurofins (Centennial, CO, USA), and the sequences were Blasted to GenBank database (https://blast.ncbi.nlm.nih.gov/Blast).

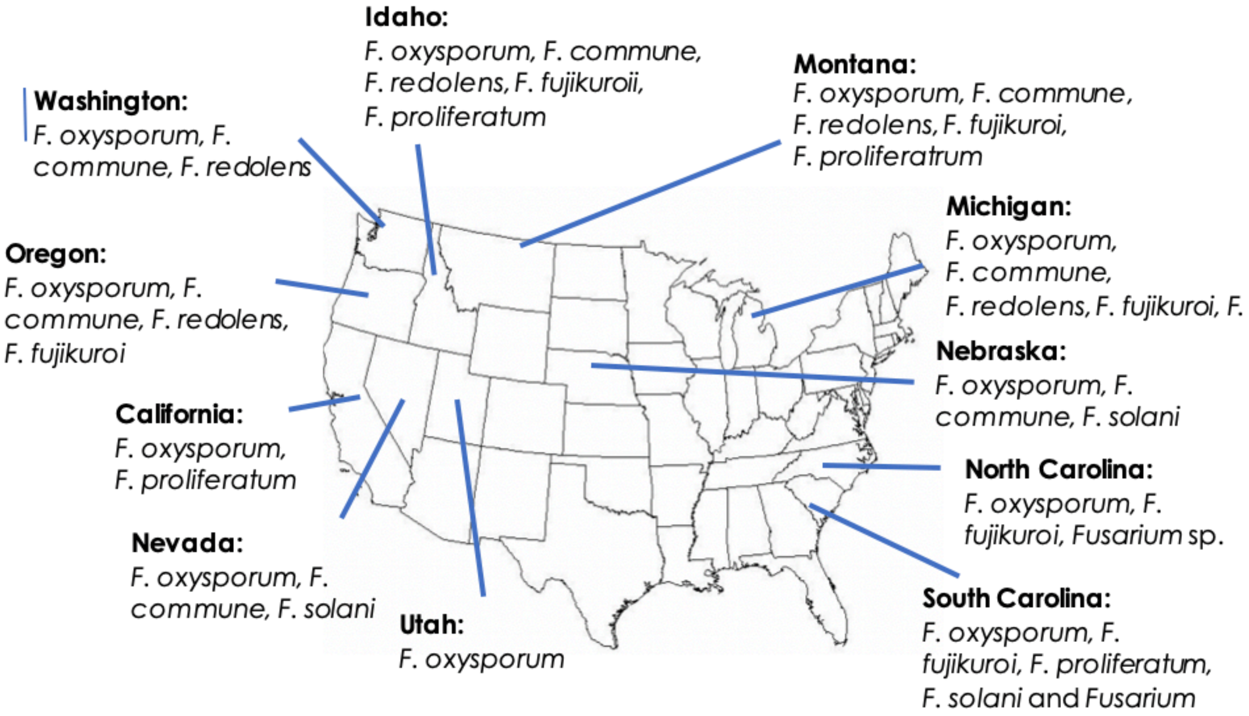

***Figure 1:*** Fusarium *species found in each state.*

## Results and Discussion

Seven species of *Fusarium* were identified within conifer nurseries in the western, mid-western, and southern states using genetic data from the mtSSU and *tef1*regions. The most diversity was observed in Idaho, Montana, Michigan, and South Carolina where at least five species were identified. The least diversity was observed in Utah, where *F. oxysporum* was identified from the one nursery sampled. Furthermore, although a total of 45 isolates were collected from five nurseries in California, 40 isolates were *F. oxysporum*, and only one was *F. proliferatum*. *Fusarium commune* was identified in Washington, Oregon, Nevada, Idaho, Montana, Michigan, and Nebraska (Kim et al. 2012). *Fusarium redolens, F. fujikuroi,* and *F. solani* were also identified from most states. Interestingly, an unidentified *Fusarium* species was identified in North Carolina and South Carolina. Further sequencing of the RNA polymerase II (RPB2) region is warranted for identifying these isolates. Understanding the pathogenicity and host range of each *Fusarium* species is important to combat damping off in conifer tree nurseries, and more intensive studies are needed to better characterize the distribution of *Fusarium* species in these nurseries.

***Table 1:*** *Count of* Fusarium *spp. isolates collected from each state (total n=350).*

| State | Total number of isolates |
|---|---|
| CA | 46 |
| ID | 56 |
| MI | 5 |
| MT | 14 |
| NE | 7 |
| NV | 13 |
| OR | 87 |
| NC | 42 |
| SC | 37 |
| UT | 12 |
| WA | 31 |

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# *ARMILLARIA ALTIMONTANA*, A POTENTIAL NATURAL BIOLOGICAL CONTROL AGAINST ARMILLARIA ROOT DISEASE

**Marcus V. Warwell[1], John W. Hanna[1], Geral I. McDonald[1], Mee-Sook Kim[2], Bradley M. Lalande[3], Jane E. Stewart[3], Benjamin C. Bright[1], Andy T. Hudak[1], and Ned B. Klopfenstein[1]**

## Introduction

Root diseases are a primary cause of reduced forest productivity, decreased carbon sequestration, and impaired ecosystem function (e.g., Lockman & Kearns 2016). *Armillaria altimontana* and *A. solidipes* (as *A. ostoyae*) are soil fungi that frequently co-occur (McDonald et al. 2000) and are found in association with western white pine (*Pinus monticola*). *Armillaria solidipes* is well known as a virulent primary pathogen on diverse conifers within inland western regions of the contiguous USA (e.g., Lockman & Kearns 2016). *Armillaria altimontana* is often considered as a weak or secondary pathogen (Brazee et al. 2012). However, little has been documented about the impact of this species on tree health and growth in nature. The objective of this study were to i) identify *Armillaria* isolates associated with each planted western white pine at the species and genet (vegetative clone) level; ii) describe their demography and spatial distribution; and iii) assess the potential influence of each *Armillaria* species on tree growth and survival of western white pine.

## Methods

A provenance test of western white pine was planted in 1971 on a 0.8-ha north-facing slop at 970 m.a.s.l at the Priest River Experimental Forest in northern Idaho, USA. The test was designed to examine variation in growth and survival among elevational seed sources in the region (Figures 1 and 2A). In 1987, 2076 living and recently dead trees were measured (e.g., survival, height, and diameter) and inspected/sampled for colonization by *Armillaria* (Figure 2B). Surveys for mycelial fans, wood root, and resinosis were also conducted in 1987. The root collar and major lateral roots of all remaining trees were inspected for *Armillaria* by excavating 0.3-m deep to a distance of ca. 1-m away from the tree base in three cardinal/ordinal directions. Rhizomorphs, mycelial fans, and rotten wood were used as samples to establish isolates in culture following the methods of McDonald et al. (1987). All isolates were paired against each other to delineate genets using the techniques described in Wu et al. (1996). A final round of diploid-diploid pairings with representatives of each genet were paired against known testers to identify species. Species identification were confirmed using translation elongation factor 1-α (*tef1*) sequences.

## Results and Discussion

*Armillaria* was found in association with 54.9% of the 2076 trees and comprised two species, *A. altimontana* and *A. solidipes*. Based on the spatial distribution of *Armillaria* species and genets and their estimated growth (van der Kamp 1993), *Armillaria* species are estimated to have occupied the site for at least 250 years (Figure 1). *Armillaria solidipes* was uncommon in areas dominated by *A. altimontana* (Figure 1). Trees colonized only by *A. altimontana* exhibited the highest growth and survival and low incidence (1.8 %) of Armillaria root disease (Figure 2). *Armillaria solidipes* was frequently (74.7%) associated with Armillaria root disease and trees colonized by *A. solidipes* exhibited the lowest growth and survival (Figure 2).

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These findings support the hypothesis that *A. altimontana* is typically non-pathogenic and may function as a beneficial symbiont, epiphyte, or both in western white pine. Furthermore, based on colonization patterns, *A. altimontana* appears to protect western white pine from Armillaria root disease by competitively excluding pathogenic *A. solidipes* in the soil (Warwell et al. 2019). The results support the possibility of new approaches for natural control of Armillaria root disease through management that encourages *A. altimontana*. Further research is needed to determine if, how, and when *A. altimontana* may be beneficial for tree survival and growth. Studies at this site using a metagenomics approach are currently underway to determine soil properties and soil microbes associated with beneficial *A. altimontana* versus virulent *A. solidipes* (Warwell et al. 2019).

***Figure 1:*** “Armillaria *species and genet distribution within the Ida Creek plantation (0.8 ha) at the Priest River Experimental Forest, Idaho, USA. Each square pixel represents 1.2 m x 1.2 m (a single tree location) and colored pixels indicate trees associated with* Armillaria altimontana*,* A. solidipes*, or both. Cells split diagonally show the two unique genets that were identified on that single tree. Grey pixels indicate trees where neither* A. altimontana *nor* A. solidipes *were found or trees that were either missing or died early from unknown causes” (Warwell et al. 2019).*

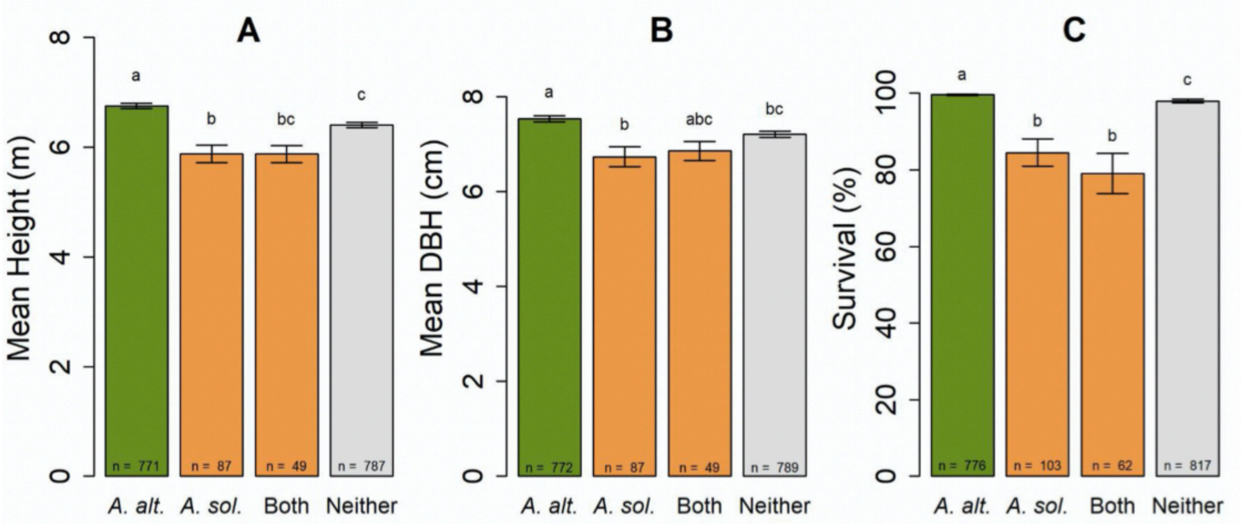

***Figure 2:*** *“(A) mean height, (B) mean diameter at breast height (DBH), and (C) percent survival among western white pine (*Pinus monticola*) growing in association with* Armillaria altimontana *(A. alt.),* A. solidipes *(A. sol.), both* A. altimontana *and* A. solidipes *(Both), or neither* Armillaria *species (Neither) at 16 years post-planting at Priest River Experimental Forest, northern Idaho, USA. Height and DBH measures were not available for every live tree in the data set. Bars show standard error. Means sharing a lower-case letter within each bar graph (i.e., A, B, and C) are not significantly different (p < 0.05) by Tukey-adjusted means separation” (Warwell et al. 2019).*

## Acknowledgements

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# COMMITTEE MEETING REPORTS

## FOLIAR AND TWIG DISEASES MEETING

### Committee Chair: Harry Kope

The breakfast meeting took place on Tuesday, June 4th from 7:00-8:30am. Harry welcomed the participants and described briefly that Foliage and Twig Disease topics were the focus of this committee, but stem diseases could fit into this discussion as well, within the twig category so to speak.

It was also pointed out that the Foliage and Twig disease committee was suggested at the business meeting as a new committee at the Sedona AZ WIFDWC in 2007, and that the first committee meeting was held in Missoula MT in 2008. After chairing the committee for 11 years (albeit not participating as chair at each meeting), Harry has asked that someone else take on the committee leadership role starting in 2020.

Topics discussed during the Twig and Foliage and Stem Committee meeting

- <u>Salal die-back on the west coast of Alaska and British Columbia - **Harry Kope**</u>
    - Robin Mulvey (Forest pathologist – Region 10- US Forest Service) – with a message relayed via Paul Hennon (retired forest pathologist) has reported salal (*Gaultheria shallon)* die-back occurring in mid to late spring along the southern Alaska coast.
    - Harry Kope and others in BC had also been made aware of the salal die-back being noticed in mid to late spring.
    - Harry Kope presented an online news video of the damage.
    - The die-back kills leaves and stems of the plants.
    - The dieback presented very quickly, almost suddenly.
    - In some areas, large patches of salal death were recorded.
    - There were strong, cold, and dry outflow winds (from the interior to the coast) in the month of February 2019. This was suggested as a possible cause.

- <u>Cedar Leaf Blight (*Didymascella thujina*) – **Harry Kope**</u>
    - Harry presented a short update on cedar leaf blight.
    - One of the issues with the disease is that when it is planted in a plantation, the 1- or 2-year-old seedlings will express the disease. The question from plantation owners was where did the disease come from?
    - To answer this question, it was described that the disease cycle of cedar leaf blight can be easily determined; the disease infects in year one and expresses in year two, therefore when the disease is noted on new seedlings it can be determined whether the disease came from surrounding stands or as infected nursery plants.

- <u>Attendance at a recent IUFRO meeting on Shoot, foliage and Stem diseases in Florence, Italy May 2019 – **Gabriela Ritokova**</u>
    - Presentation at the IUFRO meeting by Patrick Bennett – population genetics analyses reveal center of origin and global introduction pathways of the Douglas-fir Swiss needle cast fungus, *Nothophaeocryptopus gaeumannii*
    - Presentation at the IUFRO meeting by Gabriella Ritokova – Everything you always wanted to know about Swiss needle cast (But were afraid to ask).
    - Cypress Canker – *Seridium cardinale* on native Mediterranean cypress

- Hemlock dieback with stem cankers in Washington - **Will Littke**
    - Unknown what is causing these cankers that attack all age classes of trees
    - The trees are in areas of glacial deltas. There appears to be high likelihood of drought; in fact there were long episodes of drought (greater than 60 days through August and September). This may be a predisposing factor.
    - Gerry Adams recognized something like this from his work in Alaska

- *Populus trichocarpa* and *Sphaerulina musiva* – **Jared LeBoldus**
    - Cankers caused by *Sphaerulina musiva* (teleomorph: *Mycosphaerella populorum)* on hybrid poplars
    - Also, the same cankers were found in native stands of *Populus trichocarpa*
    - An interesting finding from looking at different isolates of the disease was represented in a graphic that showed a phylogenetic tree with three clusters
        - One cluster representing the south east of the US
        - One cluster representing the north central plain of the US and BC
        - One cluster representing south central BC
    - The cluster from south central BC is unique from the other cluster, and it is the same disease that was found in these Oregon stands.
    - The question was asked how did the fungus move between these two areas – and it was suggested that people unintendedly move the disease on cuttings between areas.

Jared LeBoldus has agreed to be the chair for the Twig and Foliage Committee starting in 2020

## RUST COMMITTEE MEETING

### Committee Co-Chairs: Jane Stewart and Paul Zambino (not present)

The rust committee took place Tuesday, June 4th during lunch, was chaired by Jane Stewart, and had about 35 people in attendance. There was a round table discussion focused primarily on Comandra blister rust (CBR) and white pine blister rust (WPBR). Reports and discussions are summarized below.

Jim Blodgett talked about a recent wave year of CBR (on lodgepole) in Bighorn and Shoshone National Forests, and of course, WPBR is still an issue. In a recent limber pine planting in the Black Hills, there was 97% survival rate. Also, historically he has conducted roadside and field plot surveys. However he decided to drop roadside plots because his data indicates that they are not as informative as the field plot data. In these plots, he has found that CBR impacts all age classes and that dwarf mistletoe is primarily found on the larger trees.

Paul Hennon talked about hemlock leaf rust and salmonberry leaf rust in Alaska that Robin Mulvey has found in Alaska.

Michael Murray mentioned that limber pine will likely be added to Endangered Species List in Canada soon. White bark pine is already listed. He also highlighted the High Five meeting in Canada the following summer.

Meg Dudley presented results of a survey of 5-needle pines in southern Colorado and northern New Mexico focusing on the Rio Grande, San Juan, and Carson National Forests conducted in collaboration with Kelly Burns. A total of 25 plots were installed in 2018 on the Rio Grande National Forest and San Luis Valley. A WPBR-infected *Ribes* plant was found in one plot for the first time in a new location on the west side of the valley, but no infected trees were identified. This is the furthest west distribution in CO. White pine blister rust infections were also identified on two other sites. At one of those sites, both limber and bristlecone pine were infected. This is only the second place that infected bristlecones have been found in nature. The other location is about 25 miles north in the Great Sand Dunes National Park.

Jane Stewart and Kelly Burns talked about limber pine survey work that included 80 plots in northern Montana to northern Colorado, with another 30 plots in Sangre de Cristo plots. Overall, over the last ten years, over 20% of the limber pines died from Mountain Pine Beetle (MPB) and/or WPBR, and 20% were declining. On many of the sites, MPB was a significant driver of mortality. At Pole Mountain in northern Colorado, however, WPBR was the main mortality agent of trees. Interestingly, incidence stayed consistent at 30% over the 10-year time frame. The biggest increase was observed in the Sangre de Cristos where incidence increased from 9% to 30% over the 10 years. Though incidence did not change, disease severity increased significantly, going from 2.9% to 6.9%.

Jane Stewart also talked about a Loop Mediated Isothermal Amplification (LAMP) for WPBR developed based on sequencing data from the Hamelin lab to test cankers and leaf tissue. This assay is needed for identification of rust on *Ribes* and on cankers when the fungus is not fruiting.

Also, a hybrid, similar to the one found in British Columbia on limber pine between CBR and WPBR, was identified on lodgepole pine in Wyoming. We do not know yet what this means in terms of pathogenicity on hosts.

Josh Bronson highlighted that Ellen Goheen recently published a Management Guide for Western White Pine. He also emphasized that the Dorena Genetic Resource Center is currently conducting resistance trials for all native 5-needle pines against WBPR, but that they also have shoot blight and *Phytophthora* issues.

Christy Cleaver discussed a project monitoring long term plots in Montana. She is currently analyzing data and will likely have results by the next WIFDWC

The last topic was focused on white pine species recovering after the establishment of WPBR. Ned Klopfenstein mentioned Idaho and other places where WPBR is difficult to find on some sites. He suggested that the western white pine population is recovering on some sites. Martin MacKenzie then mentioned that western white pine is becoming sterile from blister rust, when a canker on the main stem causes mortality in the top of the trees (spiked topped). Anna Schoettle suggested that this is observed in whitebark pine as well. There was discussion if there are long term plots to examine whether in some areas tree are recovering. Ned mentioned that in Idaho there are plots that were examined in 1967 that we could go back to. Christy Cleaver then mentioned that it might be difficult because 90% of the trees have died from selective harvesting and blister rust in northern Idaho.

## ROOT DISEASE COMMITTEE MEETING

### Committee Acting Chair: Michael McWilliams

The breakfast meeting took place on Wednesday morning, June 5. The meeting was chaired by Michael McWilliams standing in for Blakey Lockman. Brief reports were offered and are summarized below. Any errors in the reports are the responsibility of McWilliams.

Jared LeBoldus from Oregon State University discussed the re-emergence of black stain root disease as a problem in young Douglas-fir stands in southwest Oregon. Disease is so severe in some areas that young Douglas-fir stands have been liquidated, and companies are starting to plant a fir/hemlock mix or 100% hemlock. Disease is occurring as soon as one year after planting and seems to be insect vectored rather than through root-to-root spread. Will Littke offered observations on the survival of *Sterimnius* and *Pissodes* in recent stumps that have not yet died and the hazard of the practice of planting soon after harvest. In thinning units, small stumps provide habitat for the vectors. The disease is occurring on thin soils and benchtops but not just on tractor logged ground. There is a grad student with Dave Shaw that is going to be working on a landscape model for black stain risk and occurrence.

Harry Kope discussed reaction to a 2018 publication by the Canadian Food Inspection Agency (Phytopathology, 108: 1509-1521) reporting the presence of *Heterobasidion abietinum* and *H. parviporum* from environmental samples based on DNA detection techniques. *Heterobasidion occidentale* is regularly found on hemlock on the coast. A single basidiocarp of *H. irregulare* was collected in the Okanagan in 1994, but it hasn't been found since in sampling with plates with selective medium or with culturing from washings of Oregon grape leaves upon which the basiodiospores are said to land and remain. The 2018 publication reports the molecular signature of *H. parviporum*, which they identified as European in origin, was found in the water from an insect trap, and that a soil sample from around Vancouver was positive for *H. parviporum* and *H. abietinum.* There is a North American variant of *H. parviporum* reported by Worrall in Colorado and New Mexico on fir. They will do sampling in Canada this summer to detect species of *Heterobasidion* that may be present. A wind sock with a fine meshed slide will be used for sampling for spores. The slide is collected periodically and tested for spores using visual or DNA techniques.

Nicolas Feau discussed mortality in western white pine seed orchards resistant to the white pine blister rust that is apparently caused by *Phytophthora cryptogea* and *P. cactorum*. Often western white pine R-genotypes are grafted on white pine rootstocks of unknown provenance and origin. Orchards were examined where trees had gone from green to yellowish in about 6 months, and some signs of *Phytophthora* infection were found. Soil baiting and isolations from roots resulted in recovery of *Phytophthora.* When white pine is artificially infected by *P. cactorum*, there is little effect for about 6 months, then mortality follows very quickly. There was general discussion of similar problems at other western white pine seed orchards, usually related to water providing good conditions for *Phytophthora* spread either from local topography or too much irrigation. The question of where these *Phytophthoras* come from is unknown, but it is very possible to acquire new species from movement of plants with soil or soil on equipment.

Michael Murray reported on a survey of four stump removal trials. Several analyses were done looking at height and diameter growth and mortality following stumping. Height and diameter growth were slightly lower in stumped areas, but the survivorship was better when stumped. Survival was 74% versus 54% with no stumping, corresponding to a 22% to 28% higher basal area in stumped areas. 3/4 of areas stumped had less *Armillaria*. The Canadian Forest Service has many of these trials and stumped areas that are now not being monitored as the folks who set them up have retired, and Michael is going to try and measure and report on them as he has time. Cruikshank did a cost effectiveness analysis on one area, and it's economically worth the treatment on productive sites. Michael would like to remeasure more of the stumped areas to get a more complete picture.

Jim Blodgett reported on some forest health surveys in Region 2. He found *Armillaria sinapina* and *A. gallica* in 75% of paper birch stands, causing about 1.7% mortality per year. In white spruce stands it's mostly *A. solidipes (ostoyae)* in combination with a spruce ips that is causing the mortality, with typical Armillaria root disease symptoms of resin production at the base of the tree. Both of these types of stands are non-commercial, 'stagnant' stands. *Armillaria solidipes (ostoyae)* is considered a non-aggressive pathogen on birch.

A west-wide survey of root diseases is taking place, and the researchers would like samples. Samples of root diseases should be sent to Moscow (Ned Klopfenstein) or Corvallis (Mee-Sook Kim) for isolation and identification. Contact them for instructions on sending samples and permits.

There was a little discussion on *A. altimontana* possibly being protective against infection by *A. solidipes*. Ned thinks disturbance may give *A. solidipes* the upper hand, but *A. altimontana* is a better saprophyte and eventually gets the upper hand. Kendra Baumgartner reported *A. altimontana* (as NABS X) on red fir in California but doesn't mention the host condition.

Lori Winton had a brief report on *Fomitopsis pinicola* (now *mounceae)* in live trees. Robin Mulvey in southeast Alaska and Lori further north are finding it as a buttrot killing live trees. It was present in many trees in a blowdown area. Alex Woods mentioned they have seen it causing mortality in central BC also. It was suggested that *F. pinicola* might be doing more than we thought in live trees, and additional observations are needed.

# DWARF MISTLETOE COMMITTEE MEETING

## Committee Chair: Dave Shaw

The WIFDWC Dwarf mistletoe committee had a good meeting, reasonably well attended. We discussed new publications (see list below), the upcoming IUFRO working group 7.02.11 Parasitic Flowering Plants in Forests meeting in October 2019 at the IUFRO World Congress http://iufro2019.com/ in Brazil, the importance of the International Parasitic Plant Society http://www.parasiticplants.org/, which has a great newsletter called The Haustorium, and finished with a round robin about projects and activities concerning mistletoes. Recent survey work by Jim Blodgett was notable. One new MS project has been initiated at Oregon State University by Stephen Calkins concerning how western hemlock dwarf mistletoe influences crown structure and function of host trees. Chris Lee and Dave Shaw have initiated a re-survey of *Viscum album*, the European mistletoe, in Sonoma County, California where it was introduced by Luther Burbank around 1900. Finally, Robert Mathiasen is working on a new field guide to mistletoes of North America, north of Mexico.

### List of new publications/research on dwarf mistletoes and other western mistletoes:

### Journal Articles

Martha González-Elizondo, Mónica Yazmín Flores-Villegas, Rebeca Álvarez-Zagoya, Socorro González-Elizondo, Marco A. Márquez-Linares, Sergio Barraza-Quiñonez, Brian E. Howell, and Robert L. Mathiasen. 2018. Effects of Mexican dwarf mistletoe (*Arceuthobium vaginatum subsp. vaginatum*) on the growth of *Pinus cooperi* in Durango, México–A case study. *Forest Pathology, 49(1): e12473. https://doi.org/10.1111/efp.12473*

Robert L. Mathiasen. 2019. Susceptibility of red fir and white fir to fir dwarf mistletoe (*Arceuthobium abietinum*) in California. *Forest Pathology, 49(3): e12516. https:// doi.org/10.1111/efp.12516.*

Robert L. Mathiasen, Shawn C. Kenaley, and Jared M. Scott. 2018. *Arceuthobium microcarpum* (Viscaceae): morphological evidence for continued species recognition and discrimination from *Arceuthobium campylopodum*. *Phytologia 100(1): 71-90.*

Robert L. Mathiasen and Shawn C. Kenaley. 2017. Arceuthobium tsugense (Viscaceae): four subspecies with contrasting morphologies and host distributions. *Journal of the Botanical Research Institute of Texas 11(2): 363-390.*

Robert L. Mathiasen and Shawn C. Kenaley. 2017. Contrasting perspectives on the measurements and taxonomy of *Arceuthobium* (Viscaceae): a long-standing controversy. *Phytologia 99(2): 95-110.*

Howard M. Hoyt, William Hornsby, Ching-Hsun Huang, James J. Jacobs, and Robert L. Mathiasen. 2017. Dwarf Mistletoe Control on the Mescalero Apache Indian Reservation, New Mexico. *Journal of Forestry 115(5): 379-384. https://doi.org/10.5849/jof.16-049*

Thomas J. Parker, Carol L. Chambers, and Robert L. Mathiasen. 2017. Dwarf mistletoe and breeding bird abundance in ponderosa pine forests. *Western North American Naturalist 77(1): 40-50. https://doi.org/10.3398/064.077.0106*

Scott M. Ritter, S.M., C.M. Hoffman, J.E. Stewart, and Tom Zimmerman. 2018. The influence of prescribed crown fire on lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) populations 33 years post-fire. *Forest Pathology 48(3): e12419. https://doi.org/10.1111/efp.12419*

## Book/Chapters

David C. Shaw, Robert L. Mathiasen, and Jonàs Oliva. 2018. Parasitic plants of conifers. Pages 134-146 in E.M. Hansen, K.J. Lewis, and G.A. Chastagner (eds), *Compendium of Conifer Diseases* (2nd Edition). American Phytopathological Society Press, St. Paul, MN. 224 p.

## USFS Reports, Monitoring

J.T. Blodgett, K. Allen, K. Schotzko, A. Dymerski, and A. Chambers. 2019. *Plot Survey of Dwarf Mistletoe and Comandra Blister Rust Diseases in Lodgepole Pine on the Shoshone National Forest: 2018*. Biological Evaluation RCSC-19-02.

J.T. Blodgett, K. Allen, K. Schotzko, and A. Dymerski. 2019. *Plot Survey of Dwarf Mistletoe and Comandra Blister Rust Diseases in Lodgepole Pine on the Bighorn National Forest: 2018*. Biological Evaluation RCSC-19-01.

B.W. Oblinger. 2017. *Incidence and severity of limber pine dwarf mistletoe (Arecuthobium cyanocarpum) on whitebark pine (Pinus albicaulis) at Newberry Crater*. Report: COFIDSCI7-01, USFS, Forest Health Protection, Central Oregon Service Center, Bend, OR.

G.J. Reynolds. 2018. *Mapping the distribution and incidence of southwestern dwarf mistletoe on ponderosa pine in New Mexico national forests using roadside surveys.* Phytopathology 108(10S), S1.85.

*Arceuthobium cyanocarpum* on *Pinus albicaulis* at Crater Lake, Oregon

# HAZARD TREE COMMITTEE MEETING

## Committee Chair: Kristen Chadwick

### Business item: Location and date for the next Western Hazard Tree Workshop

The Western Hazard Tree Committee is searching for a new location and venue for the next workshop. One was scheduled for October of 2019 in Missoula, Montana. However, the committee decided to postpone it for a year due to the local FHP pathologists taking positions elsewhere. Therefore, the committee is looking for possible locations for the next meeting with a date that will likely be October of 2020. Dan Omdal suggested possibly hosting it out of eastern Washington in the Wenatchee-Leavenworth area. Kelly Burns and Jim Blodgett offered up R2 with a possibility of doing it out of Cody, Wyoming. Kristen will work with the planning committee and the local pathologists for these areas to explore both options. R5 is willing to host it in California when the next meeting rolls around.

Update: as of December 2019, the meeting will be hosted out of Wenatchee, Washington October 20-22nd. There is ~$8,000 in the budget to start the planning for the meeting, and a contract has been signed with the Hilton Garden Inn in Wenatchee.

### Special presentation: Jim Blodgett

Rocky Mountain Region's Tree Failure Forms

USDA Forest Service, Rocky Mountain Region, Forest Health Protection, 8221 Mt Rushmore Road, Rapid City, SD, USA

An International Tree Failure Database (ITFD) committee was organized to bring back the ITFD; funded under the USDA Technology Grants. Their objectives are to develop electronic forms that can support real-time database updating and to consolidate all previous ITFD data. The plan is to develop customizable forms for use in the Survey123 app by ESRI. Working with Dudley Hartel, the grant principal investigator, USDA Forest Service Region 2 (Rocky Mountain Region) developed tree failure evaluation forms for documenting tree failures in developed recreation sites on national forests. In Region 2, the *e*-forms are organized in ArcGIS groups by regional forest. Forests manage their own data and forms have customized host tree lists. Although the data is not directly shared with the ITFD, it is 100% compatible with the proposed new ITFD, and sharing data is encouraged. The Region 2 versions include only fields common in developed recreation forest sites. The idea was to simplify the forms so they were less intimidating to users. The forms can be run on any smartphone, tablet, or computer using the Survey123 app. The Region 2 forms were completed in March.

Hazard Tree Surveys and digital data collection: Jim’s presentation brought up a lot of discussion about data collection and forms for documenting hazard tree surveys. Lori Winton presented R10’s digital data collection methods at the Sitka WIFDWC. This lead to discussion on differences between the old ITFD, focusing on documenting tree failures, compared to more recent regional efforts to develop an electronic platform for hazard tree surveys. One issue with having a west wide global digital data collection method is that each region has a different rating system based on their field guide. R10 currently uses Survey123 and R6 uses Collector.

## Special Presentation: Sarah Navarro

Sarah presented on a unique tree at Cape Lookout State Park Campground in Oregon. During a training session with Oregon State Parks, with Blakey Lockman and Kristen Chadwick assisting, they found a large Sitka Spruce with conks at about 25 feet on opposite sides of the trunk. There were also multiple *Porodaedalea pini* conks present. They couldn’t determine if the conk was from *Fomitopsis pinicola* or *Laricifomes officinalis*, even with three pathologists present! Luckily, one of the OSU students had his climbing gear with him. He climbed it and removed the conk. It was a *F. pinicola* conk, and on the back side was evidence of an old *P. pini* conk. It is thought that *F. pinicola* was fruiting out of an old branch stub where *P. pini* had previously been fruiting. ODF and Oregon State Parks jointly share a resistograph. Due to the complex nature of the tree and the unusual finding of *F. pinicola*, the climber used the resistograph at the location to determine sound rind thickness (nothing like taking an $8,000 piece of equipment up a tree!). The section of decay from *F. pinicola* was not significant and the decay from *P. pini*, of which we typically don’t see failures from, was well within sound rind thickness guidelines. The tree is located in a campsite just off the coast and is one of the more heavily used sites, in addition the campground is open year-round.

# CLIMATE CHANGE COMMITTEE MEETING

## Committee Chair: Alex Woods

The committee meeting started off with prepared talks from Gerry Adams, Will Littke, Danny Norlander, and Alex Woods.

### Gerry Adams

Gerry presented an example of how long-term weather data and dendrochronological analysis can be used to show long term relationships between weather events and tree rings. At his study area in Nebraska, they are particularly interested in drought stress on native tree species, particularly species of value as choices in urban forests, parks, and landscape. Nebraska has an almost linear precipitation gradient east-to-west going from mesic to semi-desert and drought is the concern for our westernmost forest stands and urban forests, as it is drought and/or fire which has delimited native tree species, such as bur oak and common hackberry, in their western range. Sample trees at the westernmost range of common hackberry provided an opportunity to examine the relationship between a host tree species and its environment threshold. The trees could only survive in areas where they were sheltered from the wind and sun.

Gerry used 100-year time series of dendro data and ran multiple regressions of mortality using software and online tool kit of Dr. David Meko at the Laboratory of Tree Ring Research at University of Arizona. Climate factors such as temperature, precipitation, and ocean oscillations can be represented in time series, monthly, seasonally, and annually. Tree growth response to climate can also be represented as time series, generally annually but can be seasonal through early and late season wood deposition. Gerry found that the Arctic Oscillation appeared to have a major influence on the growth of hackberry in its westernmost range. June temperature and precipitation were the strongest drivers of radial growth for preceding and current years. Previous year’s April and May temperature were also positively correlated with radial growth in response function analysis.

The presentation led to a broader discussion about the differences between modelled weather using PRISM data and on-site local weather stations. There is a need for more microsite level weather information, but the use of data sources like PRISM data can help us investigate larger scale phenomenon in our rapidly changing environment.

### Will Littke

Will described a sudden widespread expression of tree and understory plant decline in the PNW. In 5-7 year-old Douglas-fir he observed widespread Phomopsis canker but he also saw extensive damage in salal and swordfern. He also observed increased rates of mortality in western hemlock of all age classes. Will suggested the damage was the result of a combination of weather events with the area experiencing a prolonged drought through summer and fall of 2018 and then a cold dry snap in February 2019. The damage appeared to be the result of combined stresses from drought and cold injury. Will was clearly concerned by this rapid deterioration in general forest health condition. The PNW received no measurable precipitation from the end of May to August of 2018, but 2017 was not much better and since 2011, Will has observed a trend of increased swordfern mortality over the longer term. The death of swordfern in these forests represents a significant change in soil water availability. The damage was most pronounced in the post glacial outwash parent materials. The role of parent materials must be taken into consideration in climate change impacts on forest species. Will said these observed changes are only forecasted to get worse with mild, moderate, and severe decreases in yearly soil water availability of 25, 28, and 53%, respectively depending on the parent materials of the soil. There has already been mortality in western hemlock and Douglas-fir based on the changing climatic conditions to date, making the effects of the forecasted climatic changes out to 2080 very worrying. “We better get ready for some real mortality”.

The environmental conditions Will described, those that led to understory species dying in typically wetter coastal forests, were also experienced along the entire length of the BC coast over summer 2018 and winter 2018/2019. Salal (*Gaultheria shallon*) was suffering dieback and mortality, and the phenomenon was picked up by local news media as the general public was clearly concerned about what was happening in their forests.

**Danny Norlander**

Danny presented a summary of his work examining the relationships between western gall rust wave years and climatic trends. He aged galls on 20-year old ponderosa pine and was able to identify wave years starting in 1996 then again in 2005. Danny looked at the possibility of a relationship between wave years and the El Niño climatic phenomenon because 1996 was one of the strongest El Niño events to date. Danny found there was an influence from El Niño but it depended on the productivity of the site, and only on the more productive sites did the relationship hold up. The highest peak in gall infections coincided with the strongest El Niño aftermath in 1997/98. Danny also found a signal in gall initiation in association with the 2015/16 El Niño, the strongest El Niño to date. El Niño events help describe about 1/3 of seasonal variations in weather with other sea surface temperature factors including the Pacific Decadal Oscillation (PDO) and other currents describing the remainder. More recently, “The Blob”, a 3° temperature anomaly in the surface waters off the Pacific coast, has been responsible for changes to weather patterns as have changes in the jet stream, and Danny discussed the possibility of these weather drivers influencing rust dynamics (Gerry Adams said the Arctic Oscillation is an important weather driver in the US mid-west; different areas have different underlying weather drivers).

In addition to the relationships between weather and western gall rust, Danny also spoke to changes in Comandra blister rust behavior in relation to longer term weather trend drivers such as El Niño. The earliest reports of wave years in Comandra blister rust appear to coincide with past El Niño events. Overall, El Niño events appear to be occurring more frequently and with higher intensity, and Danny says these are “red flags” that we should be concerned about. As the climate warms and there are longer growing seasons, will that not also mean longer periods of susceptibility to fungi? There is also the question of phenology and whether fungal development stays in-sync with host plant development. Is susceptible host tissue available when the rust spores are flying?

One of the more challenging aspects of linking rust behavior to climate trends is the increasing variability of weather and climate. The longer-term trends in an area may be towards warmer and wetter conditions, but that doesn’t mean there won’t be hot droughts or conditions such as those Will Littke described where cold snaps played a significant role in the health of numerous species. Increasing climate variability and wilder extremes are very hard on plants.

**Alex Woods**

Alex described several pieces of evidence that suggest the landscape level incidence and severity of hard pine rusts (western gall, Comandra, and stalactiform rusts) have increased in the central interior of BC. He acknowledged that in order to draw such conclusions we need long term monitoring plots of all forest health agents, and we simply don’t have enough of those installations. In the early to mid-1980s, Bart van der Kamp and others considered the landscape level incidence of Comandra rust as low (<1%) but by the late 1990s that had increased to more than 12%. More importantly the combined rust incidence had increased from an estimate of only 7% of stands having more than 5% of trees affected in the late 1980s to early 1990s to more than 40% of stands having more than 20% of all pine trees affected by the mid-2000s.

Alex then described how the biogeoclimatic ecological classification (BEC) system used in BC to classify ecological conditions has been forecast to change with climate change (Hamann & Wang 2006). He used climatic data from a weather station in central BC, Fort St. James, to show how two of the most influential environmental factors for rusts have changed in the past 100 years. Days of summer precipitation have increased, but more importantly, overnight minimum temperatures in August, when the weakest link in the rust life cycle (basidiospores) are released from the alternate host plants, has increased most significantly. Overnight minimum temperature is critical for rust infections (Van Arsdel et al. 1956).

Then Alex described a more sobering situation that was more akin to Will Littke's talk, where the severe drought of 2017/2018 put all other considerations of climate change forest disease interactions into perspective. We can attempt to understand how changes in climate will affect host/pathogen interactions with the pathosystems we understand best, but when the rain is shut off like it was for the summer of 2018 in BC, all bets are off. Extreme droughts are game changers for forest health. The persistent patterns in North America that are associated with either extreme droughts or extreme precipitation are linked to the jet stream. The jet stream is driven by the temperature differential between the cold arctic and warmer southern latitudes. When the arctic warms rapidly, as it is currently, the differential diminishes so the driver of the jet stream weakens and weather patterns can stall.

**Round Robin Rousing Discussion**

Will Littke described how Douglas-fir needs both chilling requirements to set buds properly to become cold hardy for winter and 400+ degree hours of consecutive sub-42°F over nighttime. If the tree doesn't experience those consecutive cooler nights by mid-October in central WA it doesn't meet its chilling requirement, making it vulnerable to killing frosts. At the other end the trees need 700+ degree hours of temps above 42°F for cones to emerge and become pollinated. There are many pests that are coordinated to the timing of tree flowering. For Douglas-fir and other species like western hemlock, both their cold hardiness and their flowering will be disrupted in a changing climate.

So what do we do with this extra level of chaos? What is our collective best response when we see how disrupted the balance of host, pathogen, and environment is becoming? Gerry Adams said climatologists have shown for his area in Nebraska there is a long term drying and heating trend over the past 150+ years, but this summer his state is flooding and this doesn't fit those forecasts.

Mike McWilliams said one of the most important things we can do given this level of uncertainty is to moderate the level of expectations for how much fiber we expect our forests to produce, using the Swiss Needle Cast forecasts as an example. The forests are simply not going to produce as much as earlier forecasts given what we already understand about climate change impacts. Alex wholeheartedly agreed with Mike's statement.

Will Littke said in the industrial forests of WA and OR each year they harvest about 2% of the forest so if geneticists and planners start to see deviations from their planned harvest volumes they can start to correct incrementally. They don't wait 10+ years to see what is going to happen, they make small changes to genotypes that are deployed in the hopes of keeping up with rate of climate change. Danny Norlander commented that such an approach may work when you have a 35-year rotation but what do we do with

80-year rotations? He also asked what do we do when faced with a 100,000+ acre fire and the limited options currently available for reforestation afterwards? What's more important? Setting up the forest for adaptation or getting something in the ground? Alex said we need far more monitoring in conjunction with modelling because we simply can't keep up modelling efforts with the rapidly changing climate. Will said we can make more intelligent choices by combining our knowledge of soil types with aspect and soil depth and plant more drought resistant genotypes in those higher risk areas. Jim Blodgett cautioned that we can't conclude that drought will be a factor everywhere because the laws of thermodynamics are clear that if conditions are very dry in one place another area has to be wetter. Mike McWilliams stated that the timing of precipitation and whether it falls as rain or snow will have a large influence on forest health conditions. Overall the precipitation may be the same in many areas but with increased temperatures there will be increased drought due to higher rates of evapotranspiration.

Dave Shaw said what if the longer-term climate trends tell us one thing and then the shorter-term weather events reveal something else. It is the short-term weather events that are critical to plant survival. Adaptations that we make have to bring into focus both long term trends and short-term variations. In the US NE, Wyka et al. (2018) show how increased summer temps and increased summer ppt have led to more foliar disease, but that could change, much as it has in NW BC where *Dothistroma* became epidemic under wetter, warmer summers and where now rivers are at record low levels. Under that level of uncertainty and unpredictability Alex said all you can do is diversify your species and reduce your expectations knowing that on balance pests and pathogens have the advantage in a rapidly changing environment. Kristen Waring agreed that from a silviculture perspective we must diversify but not just in terms of species, we must also diversify management approaches, including stand structures, by incorporating more partial cutting. We will have to manage our expectations of future stand growth, and in some cases we may have to practice triage and give up on some things.

We discussed as a group whether or not this advocacy for different approaches to forest management was the responsibility of forest pathologists. It was suggested that a lot of the decisions regarding resource management are market driven so perhaps with more wood product studies it could be demonstrated that Douglas-fir dimensional lumber was not the only product available. Mike McWilliams picked up on the triage idea and said that at the southern limit of the Malheur National Forest the tree line was at the 14" rain threshold but that line was expected to move much further north so a bunch of tree species will no longer be able to live there. Concentrate management and reforestation efforts on the areas that have a future and walk away from those that don't. We discussed our role as pathologists in telling the story from our knowledge perspective of where we saw forests being challenged and why given what we know about what factors ultimately kill trees. Will Littke said we should also focus our limited resources to study tree declines in areas where we expect to see the worst impacts first so that we can extrapolate from there over time as the climate continues to change. We had some debate as to whether or not such an approach, focusing on the worst first, was perhaps dishonest. We came to the conclusion that with limited resources and time it was best to focus on areas where we could see things happening.

Amy Ramsey brought up the subject of longer-term bigleaf maple decline and a study a former grad student had conducted on the tree species. She found when she reanalysed the data and looked at associations with temperature and precipitation that the tree species decline correlated with increased temperatures and decreased precipitation. The discussion then swung into the veracity of PRISM data and the validity of analyses based on those data. Paul Hennon defended the PRISM climate data modelling approach, saying it was the best we had in many areas simply because we don't have many long-term weather stations with records back to the late 1800s.

Sarah Navarro mentioned her office receiving lots of calls from the public about the health of western redcedar in western OR. These are not calls about western redcedar being planted where it shouldn't have been, these are about cedar in the riparian zone, with their roots in the stream, and still they are dying. They are not sure what is going on but believe it is drought and vapor pressure deficit (VPD) driven. Dave Shaw followed up that he thinks the cedar dying is consistent with the bigleaf maple dying and that during the summer of 2018 in central OR there were three week-long periods above 90$^o$F that coincided with a 90-day long rain free period. Dave's working hypothesis is that these tree species failures are due to these hotter droughts and cavitation events. Using PRISM data, the VPD during 2015-2018 was 15-30% higher in July and August than the longer term average over the period 1995-2010. Dave said all climate change models are projecting VPD to increase dramatically. Sarah said their group is advising forest owners to look at NRCS soil data to look at drainage and soil characteristics as these will drive what happens to tree species. Sarah is seeing whole tree mortality in western OR, not just top dieback. Paul Hennon said similar reports of cedar dieback were coming in to Robin Mulvey in SE AK, mostly top dieback, not whole tree mortality.

We were all reminded that we are currently early on in climate change projections and we are already seeing so many examples of adverse effects. If $CO_2$ emissions and subsequent climate change trajectories continue as they currently are it is not hard to see how adverse impacts will get much more severe. Our role as pathologists is to ensure that message gets through to the people who are forecasting how fast the forests are growing. Anna Schoettle said we have to get the message of reduced expectations in forest growth forecasts but also dispel the idea that somehow we will be able to fix it. We are going to have to live with more uncertainty. Large scale monitoring is one of the few proactive steps we can take to address the unprecedented levels of uncertainty we face. Sarah Navarro said there is a potential role for citizen science in that monitoring to bring in more observers and observations. Our role as pathologists is to interpret what we see in the large-scale monitoring informed by our knowledge base. Danny Norlander said we need to get involved in the policy discussions as we have important knowledge of the interactions between forest pathogens, forest health, forest management and climate change that needs to be shared.

## Literature Cited

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# NURSERY PATHOLOGY COMMITTEE MEETING

## Committee Chair (Acting): Josh Bronson

The nursery committee meeting took place on Friday morning and was well attended. There were no formal presentations so a majority of the meeting was round-robin and discussion. I began the meeting by attempting to emphasize the amount of research that is happening with *Phytophthora* species and the increasing threat of movement between nurseries and wildlands. The discussion quickly evolved into a variety of subjects related to nursery pathology.

### Round Robin

Josh Bronson – Focused on *Phytophthora* and *Fusarium* in bare-root nurseries. The most likely culprits are *Phytophthora cryptogea* and *P. megasperma*; commonly found at the end of beds near standing water at JHSN. Difficult to follow seedling once they leave the nursery, many seedlings sold to other forests/BLM districts in OR and WA. JHSN is irrigated with irrigation pond water, container stock uses city water. Continued questions on native plant pathology. Looking forward to working on *Fusarium* and *Phytophthora* molecular diagnostics STDP with Mee-Sook and Jane.

Gerry Adams – Methods developed to detect diseases but not being used to verify clean stock. Not being applied. Federal nurseries need to utilize tools, especially for identifying needle cast and shoot blight fungi that may be on asymptomatic seedlings. Continued issues with nursery people continuing to use methyl bromide even though issue was solved through research and new management plan. Federal nurseries are the best place to put into practice new methods.

Will Littke – Lots of turnover causing loss of knowledge. “New” pathogens showing up that may have been managed before. Pesticides may be used incorrectly. Loss of Nursery coop created a gap in knowledge. Loss of timber extraction in 1990s led to big loss in nurseries. Reforestation practices are again becoming an important tool to address climate change and for rapid response following wildfire. Recent beetle kill emphasized need for quick recovery following disturbance. Trained people are needed, especially when using tools such as PCR to identify one of the many seed and seedling pathogens. Basamid needs to be applied correctly in order to be effective, not effective in heavy soils.

Susan Frankel – Accreditation program in CA, nurserymen required to have training once a year. Ongoing training throughout year. Developing a diagnostic guide for native plants.

Mike McWilliams – Need for operational monitoring of outplantings of nursery stock.

Jared Leboldus– OSU has Contract for Phytophthora diagnostics used for nurseries (Dorena, JHSN, etc.). Collaboration is happening. Nursery issues presented in at least two forest health classes, Jane agrees.

Jane Stewart – There actually has been a lot of work collaborating with researchers, including herself, Jared Leboldus, and Mee-Sook Kim, and nurseries. Endophytes research is increasing as they are recognized as potential pathogens.

Will – Seed pathogens used to be studied intensely in 1980s. Where seed comes from can affect pathogen populations (e.g., seed cache vs orchard collections). Important in seed transfer. Drawbacks to achieving healthy forests in future came with nurseries closing and lack of planting.

Gerry – Seed borne Diplodia work in Michigan. Found stands that are Diplodia-free. Unfortunately was not recognized as a clean seed source. Seed source can be questionable depending on where you work and who you work with.

Jim Blodgett – They only call us when there's a problem, but often don't because they are afraid what will be found. Bessey nursery container seedling planted limber pine. 98% survival. Dead from damping off *Fusarium* from nursery?

Will – *Fusarium* populations in ag lands 100 times more than other areas. *Fusarium roseum* on seed can cause lots of damping off. Thyram can be used as seed treatment and makes good bird deterrent, can cause bad reaction with people who drink alcohol. Mowing grass increasing *Fusarium* spore load.

Jane – There is funding for *Fusarium* research (John Dobbs, PhD student). Six different species causing disease on conifers, virulence is different. Working on genomes and population study of *F. commune/oxysporum*. Working on developing genome id. *Fusarium commune* not found in SE, some in Michigan.

Mee-sook Kim – Very interested in *F. commune* population distribution across U.S.

Harry Kope - BC nurseries used to be strong, but have been shut down. Using only seed from BC, no cost recovery. No money, no pest clinic. Usual characters present however no support. Nursery pathology in BC has declined and is no longer active, although interest remains.

Will – Nursery system is at the heart of all reforestation issues. Communication breakdown between the nurseries, industry and research, it's always the same. Planning years in advance not happening for seed selection, stratification and site preparation takes planning years out. That message is not reaching ecologists and land managers to get to nursery managers. Huge shipments coming in Miami airport, APHIS system is broken. Too much to inspect, too many ways to appear clean when you are not. Miscommunication in state inspection services.

Harry – *Phytophthora ramorum* update. BC BPAC discuss issues in nurseries looking for nursery transfers in province. No new detections on Vancouver Island. But introductions have been eradicated. Six nurseries (three propagation, three retail) on mainland found positive. Questions remain for pathways, a lot of trade of dirty 6 not established but originates from small samples.

Gerry – Uninvestigated pathway for *P. ramorum* in U.S. is from Europe to Canada then over the border. Rose breeders possibly just shipping cuttings and other plant materials that may come from *P. ramorum* infested areas.

Jared – Is there industry support for doing more inspections, support or funding path? USDA CAP grants? Agrobacterium multi-state project at OSU. The forest can be a source of inoculum in nursery, embellish to gather support, form argument in a certain way to get support from industry

Sarah Navarro - Fire and SOD? Not much control of *P. ramorum* in Chetco bar fire. Sanitation requirements were included from resource advisors for water management. Unfortunately a couple of instances where fire lines were drawn just beyond *P. ramorum* positive sites. Patchy nature of wildfire not useful for pathogen control.


MICHIGAN STATE
FORE

# OTHER REPORTS

# BUSINESS MEETING MINUTES, 66TH WIFDWC

**Co-secretaries: Gregory J. Reynolds and Nicholas Wilhelmi**

The WIFDWC business meeting was called to order by the Conference Chair, Kelly Burns, at 10:15 AM on Friday, June 7th, 2019. There were 64 people in attendance.

The chair thanked the meeting organizers, local arrangements, and committee chairs for the Estes Park meeting; Marianne Davenport and Monica Khazaal from R2 Forest Health Protection, Brian Verhulst from Rocky Mountain National Park, and the Jane Stewart Lab (Colorado State University) were also thanked for facilitating and supporting the meeting.

## OLD BUSINESS

A motion to adopt the WIFDWC 2017 business minutes without revision was made. The motion was seconded and passed.

## NEW BUSINESS

### Deceased Members

There were no deceased members to add since the previous WIFDWC in Parksville, British Columbia.

### Next WIFDWC

Christy Cleaver presented the meeting location for the next WIFDWC, which will be in Sandpoint, Idaho on the shores of Lake Pend Oreille in May of 2020. This location is in the northern Idaho panhandle, about 46 miles north of Coeur d'Alene. The lake is the largest in Idaho at 43 miles long and has been used for testing submarines by the U.S. Navy. Spokane, WA is the closest large city. American, Alaska, Delta, Frontier, Southwest, and United Airlines serve the airport. Ground transport to Sandpoint may be arranged through Bonner Taxi and White Tail Transportation. There is an Amtrak station in Sandpoint with westbound trains departing at 11:49 PM and eastbound trains departing at 1:40 AM. The venue will be the Best Western Edgewater Resort. A block of 35 rooms at the government rate ($94 per night) will be available. Every room has lake and mountain views. The venue is walking distance to various restaurants and breweries in the city. Other lodging options include Holiday Inn, LaQuinta, and more. Possible activities include boating, fishing, hiking Scotchman Peak (with mountain goats), and more. Employees of the U.S. Forest Service who wish to attend will need to submit their names immediately with the Attendee Detailed Cost Analysis Spreadsheet (ADCAS). Highlights of the meeting field trips will include root disease, dwarf mistletoes, stem decays, and white pine blister rust. The slate of candidates for the 2020 meeting Executive Committee was: Conference Chair (Sarah Navarro), Program Chair (Mee-Sook Kim), Local Arrangements (Christy Cleaver and Paul Zambino), Secretary (Brent Oblinger), Treasurer (Holly Kearns). Michael McWilliams made a motion to accept nominations, which was seconded and passed. Jared LeBoldus had not yet informed Greg Filip that he had been awarded the Outstanding Achievement Award and requested attendees keep this information to themselves until Greg was made aware of the award.

### Future WIFDWC

Regions 3 and 5 were identified as potential locations for the 2021 WIFDWC, because the meeting has not been in either region since 2007 and 2012, respectively. Sarah Navarro proposed Santa Rosa as a potential location with Cal Fire sponsoring; Chris Lee is interested in hosting. Jared Leboldus proposed accepting this meeting location, Kelly Burns seconded, and the motion passed.

**Committee Updates**

Gerry Adams proposed changing the Foliage & Twig Committee to the Foliage & Canker Committee. Harry Kope, Committee Chair, proposed just adding cankers to this committee unofficially so the bylaws don't need to be changed. He said that the meeting was well attended despite occurring early in the meeting. Jared Leboldus is the new proposed chair for this committee.

The rust committee, chaired by Jane Stewart, discussed the hybrid between *Cronartium ribicola* and *C. comandrae*. Christy took notes for this committee.

The root disease committee, chaired by Michael McWilliams, discussed *Heterobasidion parviporum* in Colorado and New Mexico, using wind socks for spore trapping, stump removal to reduce mortality, and whether *Fomitopsis pinicola* should be reclassified as a pathogen of live trees. Jim Blodgett also presented on *Armillaria* in hardwoods.

The hazard tree committee, chaired by Kristen Chadwick, discussed postponing the next hazard tree workshop because of the vacancies in Missoula. The workshop will likely occur in October 2020 in Cody, Wyoming or Leavenworth, Washington. Differences in rating systems between regions was discussed. Sarah Navarro shared an interesting spruce case study involving *Fomitopsis pinicola* and *Porodaedalea pini.*

The nursery pathology committee, chaired by Josh Bronson, had no formal presentations. There has been reduced interest in this area due to a decline of the nursery trade. The message at the meeting was to stay positive because the situation will improve.

The climate change committee, chaired by Alex Woods, had four speakers. A more structured meeting in the future was proposed to discuss monitoring and inserting ourselves in policy discussions.

The Outstanding Achievement Award Committee is currently composed of Jared LeBoldus (Chair), Ellen Goheen, and Anna Leon. Anna Leon will be the new chair. Alex Wood and Jane Stewart were nominated to replace Jared and Ellen.

Current student travel award committee members include Jared LeBoldus, Harry Kope, Dave Shaw, and Betsy Goodrich. Harry stepped down, and Rachael Sitz was nominated by Jane Stewart as his replacement. Dave Shaw stepped down and Kelly Burns was nominated to replace him. Hazel Daniels motioned to accept new nominations; the motion was seconded and passed. All graduate students at this meeting received a travel award: $250 for Ph.D. students and $150 for M.S. students. Sarah Navarro stepped down from managing the silent auction and Hazel Daniels was nominated to replace her with no dissent over a student holding the position. There is a need to bring more items, both expensive and cheaper. Donations to Holly Kearns for the student travel fund are appreciated because only half as much money was made compared to previous years. Forestry Suppliers can provide materials for the silent auction as donations; old publications were also mentioned as another potential item for this event. At this time, WIFDWC is not considered a non-profit.

## Website Update

Danny Norlander requested attendees send him photos, blog entries, and website feedback. New job postings may be sent to Danny and Kristen Chadwick. In the first half of 2019 the website had over 1,900 visits with over 4,600 specific page views.

## Treasurers Update

No treasurer report was prepared. In the past year, WIFDWC made some money and spent some.

## Letter of Support for WIFDWC

Michael McWilliams proposed that WIFDWC write a letter of support to the Chief of the U.S. Forest Service, all Regional Foresters, and counterparts in Canada to generate support for western pathologists wishing to attend the conference. We typically do compose a letter every year, typically written by the conference chair, but in the past this has been specifically in support of research pathologists. Inviting Bruce Moltzan or Rick Cooksey so the Washington Office would have representation could help, but that would take a spot from western pathologists. Anna Schoettle suggested that WIFDWC should be a mission operational meeting rather than a conference because it is so much more interactive than other conferences like the American Phytopathological Society. Jim Blodgett has some bullet points on the importance of WIFDWC that could be added to the letter. Sarah Navarro volunteered to take the lead on composing the letter, which will then be included in meetings management (the new conference planning) justification.

## Job Openings

Josh Bronson mentioned that Ellen Goheen's sudden oak death position will be opening soon, and an entomologist is needed in Medford as well. The Forest Health Protection pathologist position in Gunnison should be opening soon. Other future job openings include technician positions in Lakewood and Rapid City, a sudden oak death technician with Jared Leboldus at Oregon State University, and a technician position in Coeur d'Alene to replace a departing employee. Postings for Forest Health Protection pathologist positions in Ogden and Missoula recently closed.

## Oral history of WIFDWC

Danny Norlander proposed compiling some kind of oral history of WIFDWC that could include items like fireside chats and one-on-one interviews. The oral history would target retired pathologists who haven't attended in a while. In addition to being a fun exercise and a good introduction to WIFDWC for new attendees, this oral history could also help to maintain knowledge of permanent plot systems. Ellen Goheen has maintained a scientific family tree that could potentially be incorporated into the oral history as well; this was last shown at a meeting in Sitka. Ned Klopfenstein previously mentioned a potential history talk in Tahoe. Those interested in developing this oral history should e-mail Danny to coordinate.

## Social Media Account

Christy Cleaver proposed developing a social media account for WIFDWC. This has been proposed in the past but attendees were previously uncomfortable with the idea. It would potentially be an effective means for disseminating information on jobs and award applications to younger members and potential members in graduate school. Danny Norlander will discuss the development of a social media account with Sarah Navarro.

## Close

At the close of new business, a motion was made by Kelly Burns to adjourn the business meeting. The motion was seconded and passed. The business meeting ended by 11:09 AM on Friday, June 7th, 2019.

# TREASURER'S REPORT

## 66th WIFDWC

## June 3-7, 2019, Estes Park, Colorado

**Submitted by Holly Kearns**

The 66th annual WIFDWC in Estes Park, Colorado had 71 attendees including 42 regular members, 11 graduate students, 8 retirees, 8 guests, and 2 single-day registrants. The following is a summary of transactions for the WIFDWC accounts from 1/1/2018 through 12/31/2019. The WIFDWC Federal Tax Identification Number is available upon request.

| | Income / Expense | Balance | Total Account |
|---|---|---|---|
| **All WIFDWC Accounts** balance 12/31/17 | | | **$30,847.79** |
| **WIFDWC Meeting Account** balance 12/31/17 | | $14,950.24 | |
| 66th WIFDWC | | | |
| Total registration | 16,365.51 | | |
| Hotel meeting rooms, meals & breaks | -6,087.98 | | |
| Field trip transportation | -3,119.10 | | |
| Field trip supplies and snacks | -472.08 | | |
| Souvenirs | -1,552.07 | | |
| Office supplies | -484.32 | | |
| Regular member registration fees to STA Fund | -1,050.00 | | |
| Other Account Activity | | | |
| 2017 Proceedings (printing and formatting) | -2,837.33 | | |
| 2017 Proceedings mailing | -419.94 | | |
| WIFDWC.org domain name (9 year renewal) | -108.00 | | |
| **WIFDWC Meeting Account** balance 12/31/19 | | **$15,184.93** | |
| **Hazard Tree Committee Account** balance 12/31/17 | | $8,302.91 | |
| No Activity | | | |
| **Hazard Tree Committee Account** balance 12/31/19 | | **$8,302.91** | |
| **Student Travel Award Fund** balance 12/31/17 | | $1,838.00 | |
| 2019 Student Travel Awards | -1,600.00 | | |
| 2019 Silent auction proceeds | 701.00 | | |
| 2019 Regular member registration fees (42 @ $25) | 1,050.00 | | |
| **Student Travel Award Fund** balance 12/31/19 | | **$1,989.00** | |
| **International Sponsorship Fund** balance 12/31/17 | | $5,756.64 | |
| No Activity | | | |
| **International Sponsorship Fund** balance 12/31/19 | | **$5,756.64** | |
| **All WIFDWC Accounts** balance 12/31/19 | | | **$31,233.48** |

# STUDENT TRAVEL AWARDS COMMITTEE REPORT

### Jared LeBoldus, Harry Kope, and Dave Shaw

The Student Travel Award Committee gave out travel awards totaling $1,600. All students presented research summaries in the Graduate Student Flash n’ Dash session, and PhD students also presented expanded papers.

Congratulations again to the following students:

**Hazel Daniels, Bradley Lalande, Erika Dort, Jessa Ata, Stephen Calkins, John Dobbs, David Atkins, and Adrian Poloni.**

Thanks again to all of those people who donated items for the auction. Through their generous donations and the excellent participation of the attendees, the silent auction raised $701.00, significantly less than the previous meeting ($1,123.00 in 2017). In addition, there were 42 regular WIFDWC member registrations which added $1,050.00 to the student travel account, which now has a balance of $1,989.00.

Harry Kope and Dave Shaw stepped down from their roles on this committee and will be replaced by Rachael Sitz and Kelly Burns in 2020.

## WIFDWC OUTSTANDING ACHIEVEMENT AWARD RECIPIENTS

| Year | Recipient | Meeting | Comments |
|---|---|---|---|
| 2000 | Lew Roth | Kailua-Kona, HI | For pioneering work on *Phytophthora lateralis,* Armillaria and dwarf mistletoes, and for inspiration and leadership of a generation of plant pathology students and colleagues. |
| 2000 | Duncan Morrison | | 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. |
| 2002 | No award given | | |
| 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. |
| | Alan Kanaskie | | For outstanding leadership, as a practicing forest pathologist, in the management of Swiss Needle Cast. |
| 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. |
| 2008 | No award given | | |

| Year | Recipient | Meeting | Comments |
|---|---|---|---|
| 2009 | Bill Jacobi | Durango, CO | In recognition of his 30-plus years as an educator, researcher, organizer, advocate and practitioner of forest pathology. |
| | Bob Edmonds | | In recognition of his 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 | Leavenworth, WA | For leadership in the science and practice of forest pathology and for critical contributions to the management of Sudden Oak Death. |
| | Ellen Goheen | | For leadership in the science and practice of forest pathology and for critical contributions to the management of Sudden Oak Death. |
| 2012 | John Schwandt | Lake Tahoe, CA | For the energy, enthusiasm, and integrity which he has invested in the professions of forest pathology and forest management. |
| 2013 | Don Goheen | Waterton Lakes, AB | In honor of your 35 years of dedicated service to forest pathology as a researcher, leader and mentor of others. |
| 2014 | Terry Shaw III | Cedar City, UT | In recognition of broad western U.S. and international experiences, and dedicated mentoring and storytelling. |
| | Willis R. Littke | | In recognition of a valuable industry perspective, support for WIFDWC Nursery Committee, international experience, mentoring and storytelling. |
| 2015 | Brian Geils | Newport, OR | In recognition of a creative scientist with a broad range of interests, a high level of enthusiasm and curiosity, and a great guy to be with in the field. |
| 2016 | No award given | | |
| 2017 | No award given | | |
| 2019 | Greg Filip | Estes Park, CO | In recognition of a lifetime of strong contributions to forest pathology research both internationally and in the western U.S. on root diseases and various other important issues |

## WIFDWC OUTSTANDING ACHIEVEMENT AWARD MEMBERS

| Year | | Members | |
|---|---|---|---|
| 2000 | J. Byler | W. Littke | B. van der Kamp |
| 2001 | W. Littke | B. van der Kamp | 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 |
| 2014 | P. Hennon | M. Cruickshank | K. Lewis |
| 2015 | M. Cruickshank | K. Lewis | E. Goheen |
| 2016 | K. Lewis | E. Goheen | J. LeBoldus |
| 2017 | E. Goheen | J. LeBoldus | A. Leon |
| 2019 | A. Leon | J. Stewart | A. Woods |

## STANDING COMMITTEES AND CHAIRS, 1994—2019

| Committee | Chairperson | Term |
|---|---|---|
| Hazard Trees | J. Pronos | 1994—2005 |
| | P. Angwin | 2006—2015 |
| | K. Chadwick | 2016—present |
| Dwarf Mistletoe | R. Mathiasen | 1994—2000 |
| | K. Marshall | 2001—2003 |
| | F. Baker | 2004—2013 |
| | D. Shaw | 2014—present |
| Root Disease | G. Filip | 1994—1995 |
| | E. Michaels Goheen | 1996—2005 |
| | B. Ferguson | 2006—2009 |
| | M. Cleary | 2010—2011 |
| | B. Lockman | 2012—present |
| Rust | J. Schwandt | 1994, 2005 |
| | R. Hunt | 1995—2004 |
| | H. Kearns | 2006—2011 |
| | H. Maffei | 2012—2016 |
| | P. Zambino and J. Stewart | 2017—present |
| Disease Control[a] | B. James | 1995—2002 |
| Nursery Pathology | B. James | 2002—2005 |
| | K. Mallams | 2007—2010 |
| | W. Littke | 2011—2014 |
| | A. Leon | 2015—present |
| Foliar and Twig Diseases[b] | H. Kope | 2007—present |
| Climate Change[c] | S. Frankel | 2007—2008 |
| | S. Frankel & D. Shaw | 2009—2014 |
| | S. Frankel, D. Shaw & A. Woods | 2015—present |

[a] Disease Control committee was disbanded in 2002.

[b] Foliar and Twig Diseases committee was made full charter member in 2009.

[c] Climate Change committee was made full charter member in 2010.

# BYLAWS OF THE WESTERN INTERNATIONAL FOREST DISEASE WORK CONFERENCE

Passed by a vote of the Membership at the Business Meeting of October 5, 2017.

## 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 Marianna 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 attended 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% 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 chairpersons 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 nominees 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.

**2012**

**Finances**—motion passed to hire a tax consultant for WIFDWC taxes.

**Student Travel Award**—motion passed to recommend to the program chair of each meeting to allow time in the program for each student receiving a travel award to present their work.

**Deceased members** – a moment of silence or tribute will be given for deceased members.

**Regional Reports** – motion passed for the Secretary to request regional reports in a standard format prior to the meeting and distribute reports at the meeting.

**Joint Meetings with WFIWC**- motion passed for the fall 2016 Executive Committee to consider having joint meeting with WFIWC.

**2013**

**Officers**- motion passed for Kristen Chadwick to maintain mailing and member list up to date, not the Secretary as specified in the bylaws.

**Fund Raising**- motion passed to increase regular registration rates by $15 to go to student travel award.

**2014**

**Joint Meetings with WFIWC**- conference chair will send an invitation to the WFIWC chair to hold a joint meeting in 2018 at a location in the US.

**2015**

**No New Motions Passed**

**2016**

**WIFDWC Website** - Danny Norlander will investigate in conjunction with the 2017 planning committee for hosting WIFDWC 2017 website on a non-federal option. WIFDWC will invest funds.

**International Funds** - funds should be used for international travelers to attend meetings in Canada or the states, but not to fund regular Canadian/American members to attend American or Canadian meetings, respectively.

**2017**

**Fund Raising** – motion passed to raise the portion of registration fees used for the student travel awards to $25.

**2019**

**No New Motions Passed**

## PAST ANNUAL MEETING LOCATIONS AND OFFICERS, 1953—2019

| Meeting | 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 | Coeur 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 Spring, 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<br>R. Hunt |
| 30 | 1982 | Fallen Leaf Lake, CA | W. Bloomberg | W. Jacobi | E. Hansen | F. Cobb<br>J. Parmeter |
| 31 | 1983 | Coeur d'Alene, ID | J. Laut | S. Dubreuil | D. Johnson | J. Schwandt<br>J. Byler |
| 32 | 1984 | Taos, NM | T. Hinds | R. Hunt | J. Byler | J. Beatty<br>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—2019 (CONT.)

| Meeting | Year | Location | Chairperson | 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<br>T. Rodgers | | |
| 43 | 1995 | Whitefish, MT | S. Frankel | R. Mathiasen | K. Russell | R. Mathiasen | J. Taylor<br>J. Schwandt | | |
| 44 | 1996 | Hood River, OR | J. Kliejunas | J. Beatty | J. Schwandt | S. Campbell | J. Beatty<br>K. Russel | | |
| 45 | 1997 | Prince George, BC | W. Thies | R. Sturrock | J. Schwandt | K. Lewis | R. Reich<br>K. Lewis | | |
| 46 | 1998 | Reno, NV | B. Edmonds | L. Trummer | J. Schwandt | G. Filip | J. Hoffman<br>J. Guyon | D. Morrison | |
| 47 | 1999 | Breckenridge, CO | F. Baker | E. Michaels Goheen | J. Schwandt | J. Taylor | D. Johnson | | J. Adams |
| 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<br>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<br>K. Lesiw | J. Pronos<br>J. Kliejunas<br>S. Smith | | |
| 53 | 2005 | Jackson, WY | M. Fairweather | H. Merler<br>J. Guyon | J. Schwandt | K. Burns | J. Hoffman<br>F. Baker<br>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<br>B. Geils<br>B. Mathiason | | |
| 56 | 2008 | Missoula, MT | G. DeNitto | F. Baker | J. Schwandt | W. Littke | B. Lockman<br>M. Jackson | | |

Bylaws passed in 1998 WIFDWC Business Meeting identify officers as chairperson and secretary elected at annual business meeting and treasurer and historian, elected every five years.

## MEETINGS AND OFFICERS, 1953—2019 (CONT.)

| Meeting | Year | Location | Chairperson | Secretary | Treasurer | Program Chair | Local Arrangements | Historian | Web Coordinator |
|---|---|---|---|---|---|---|---|---|---|
| 57 | 2009 | Durango, CO | G. Filip | J. Adams | J. Schwandt | D. Shaw | K. Burns<br>B. Jacobi<br>J. Worrall<br>R. Mask<br>J. Blodgett | R. Sturrock | J. Adams |
| 58 | 2010 | Valemount, BC | R. Sturrock | M. Fairweather | J. Schwandt | D. Goheen | M. Cleary<br>R. Reich | | |
| 59 | 2011 | Leavenworth, WA | P. Angwin | S. Zeglen | H. Kearns | A. Kanaskie | G. Filip<br>A. Saavedra<br>A. Ramsey-Kroll<br>D. Omdal | | |
| 60 | 2012 | Tahoe City, CA | A. Woods | J. Browning | H. Kearns | P Hennon | P. Cannon<br>B. Woodruff | | |
| 61 | 2013 | Waterton Lakes National Park, AB | R. Reich | K. Chadwick | H. Kearns | B. Lockman | T. Ramsfield | | |
| 62 | 2014 | Cedar City, UT | M. McWilliams | M. Murray | H. Kearns | J. Worrall | J. Guyon | | |
| 63 | 2015 | Newport, OR | A. Kanaskie | A. Ramsey | H. Kearns | E. Goheen | K. Chadwick<br>A. Kanaskie<br>G. Filip<br>D. Shaw | | S. Romero |
| 64 | 2016 | Sitka, AK | P. Hennon | B. Goodrich | H. Kearns | H. Kope | R. Mulvey<br>P. Hennon | | B. Lilly |
| 65 | 2017 | Parksville, BC | H. Kope | C. Cleaver | H. Kearns | D. Shaw | S. Zeglen | E. Becker | D. Norlander |
| 66 | 2019 | Estes Park, CO | K. Burns | G. Reynolds<br>N. Wilhelmi | H. Kearns | J. Stewart | J. Stewart<br>K. Burns<br>J. Blodgett | E. Becker | D. Norlander |

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.

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Jesse Bedwell
Robert Bega
Warren Benedict
John Bier
Richard Bingham
Bill Bloomberg
Roy Bloomstrom
Thomas "Buck" Buchannan
Don Buckland
Hubert "Hart" Bynum
Elmer Canfield
Fields Cobb
Ross Davidson
Oscar Dooling
Charles Driver
Norm Engelhart
David Etheridge
Mike Finnis
Ray Foster
Dave French
Alvin Funk
Robert Lee 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
Neil E. Martin
Tom McGrath
Neil E. McGregor
Jim Mielke
D. Reed Miller
Alex Molnar
Vergil Moss
Harrold Offord
Nagy Oshima
Lee Paine
John Palmer
John "Dick" Parmeter
Fred Peet
Glenn Peterson
Clarence Quick
Jack Roff
Lew Roth
Keith Schea
Dave Schultz
Charles G. Shaw
Albert Slipp
Richard B. Smith
Willhelm Solheim
Albert Stage
Phil Thomas
Eugene Van Arsdel
Willis Wagener
Gordon Wallis
Charles "Doc" Waters
Larry Weir
Ed Wicker
John Woo
Ernest Wright
Wolf Ziller

1) Gabriela Ritokova, 2) Kelsey Søndreli, 3) Hazel Daniels, 4) Suzanne Marchetti, 5) Kelly Burns,
6) Jane Stewart, 7) Mee-Sook Kim, 8) Jessa Ata, 9) Martin MacKenzie, 10) Kristen Waring,
11) Gerard Adams, 12) Susanna Keriö, 13) Adrian Poloni, 14) Sarah Navarro, 15) Yung-Hsiang "Sky" Lan,
16) Jared LeBoldus, 17) Ned Klopfenstein, 18) Monica Khazaal, 19) Marianne Davenport,
20) Stephen Calkins, 21) Daniel Krieble, 22) John Hart, 23) Gail Thies, 24) Nick Wilhelmi, 25) Walt Thies,
26) Rachael Sitz, 27) Josh Bronson, 28) Jorge Ibarra Caballero, 29) Jim Blodgett, 30) Megan Dudley,
31) Christy Cleaver, 32) John Miller, 33) Michael Murray, 34) Lori Winton, 35) Diana Tomback,
36) Erika Dort, 37) Andrea Garfinkel, 38) Dave Shaw, 39) Laurel Sindewald, 40) Scott Ritter,
41) Brian Verhulst, 42) Christine Holtz, 43) Anna Schoettle, 44) Brent Oblinger, 45) Tom Eckberg,
46) Patrick Bennett, 47) Dan Omdal, 48) Greg Reynolds, 49) Susan Frankel, 50) Nicolas Feau,
51) Alex Woods, 52) John Hanna, 53) Paul Hennon, 54) Angel Saavedra, 55) Mike McWilliams