

## Systematic Study of *Nattrassia mangiferae*, the Cause of Madrone Canker

### Marianne Elliott and Robert L. Edmonds

**Abstract**—The fungus identified as *Nattrassia mangiferae*, which causes a canker disease of Pacific madrone, was studied using morphological and molecular methods. Only asexual spores were observed, but sequencing of the ITS region of the ribosomal rDNA places the sexual state in the genus *Botryosphaeria*. The fungus resembles *Fusicoccum anamorphs* of closely related *Botryosphaeria* species and has a similar pathology.

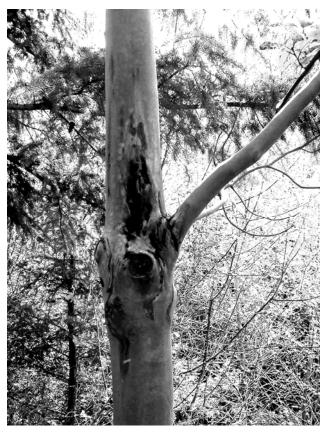
#### Introduction\_

The coelomycete fungus *Nattrassia mangiferae* has been implicated as the cause of a canker disease in Pacific madrone (*Arbutus menziesii* Pursh) (figures 1–2).



**Figure 1**—Declining madrone trees in Seattle, WA. Notice the extensive dieback in the crown and the bumpy, irregular appearance of the stems due to cankering.

Some anamorphs of *Botryosphaeria* are similar to *Nattrassia*, both in their behavior on the host plant and morphologically. They attack stressed, especially drought stressed, trees, and are primarily wound-invading. They cause canker and branch dieback, and are often endophytic, triggered to cause symptoms when the host is under drought, shade, or defoliation stress. These fungi primarily infect angiosperms but are found on some gymnosperms (*B. dothidea* on Sequoia and *Nattrassia* on pine).



**Figure 2**—*Nattrassia* cankers on the bole of a young Pacific madrone. Older cankers are sunken. Staining under the bark may be necrosis from toxins produced by the fungus.

Nattrassia is a polymorphic fungus that has two spore stages, the pycnidial and the arthroconidial (*Scytalidium* state). Sutton and Dyko (1989) revised the genus *Hendersonula* and created the new monotypic genus Nattrassia, whose type species is N. mangiferae. Earlier names for this fungus have been Dothiorella mangiferae, Exosporina fawcettii, Fusicoccum eucalypti, Hendersonula cypria, H. agathidis, and H. toruloidea. The arthric syanamorph is known by the name Scytalidium dimidiatum, also Torula dimidiata, and S. lignicola. Exosporina fawcettii was originally described as a parasite of fruit trees in California and is similar to Nattrassia isolates from madrone. A sexual stage for Nattrassia has not been described.

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#### Objective\_\_\_\_\_

The objective of this research was to determine the proper classification of *N. mangiferae* isolates from Pacific madrone. This was accomplished using cultural and molecular methods.

#### Methods\_\_\_

#### Morphology

Fifteen isolates of *Nattrassia* were grown on potatodextrose agar (PDA) plates in the dark at  $25^{\circ}$  C for one week, then moved to a temperature-controlled room at  $10^{\circ}$ C with a 10 hour light and 8 hour dark cycle for six weeks. Macroconidia and arthrospores were measured in a compound microscope with an ocular micrometer. In addition, the shape, color, and septation was noted. Presence or absence of microconidia was recorded.

#### Molecular

Frozen mycelium was extracted and diluted to a concentration of 200:1 with sterile deionized water. PCR of the ITS region was performed in a 25  $\mu$ l volume using 5  $\mu$ l DNA and the primers ITS-1F and ITS-4. PCR products were purified and sequenced in the University of Washington Biochemistry DNA Sequencing Facility on the ABI 3700 high throughput capillary DNA analyzer.

ITS sequences from closely related species (determined from a BLAST search) were downloaded from GenBank and included in the analysis. The ingroup contained 23 isolates with aligned length 538 bp. *Sphaeropsis sapinea* (AY160200) was used as the outgroup. *Nattrassia* sequences which were identical to each other were removed from the analysis.

#### Data analysis

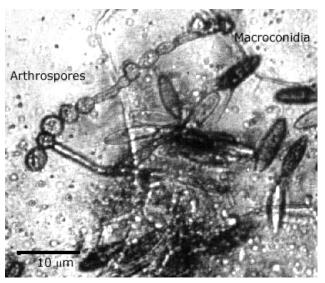
Conidial length, width, and length to width ratios, and arthrospore area (calculated using the formula for an ellipse) were compared for each isolate using 1-way ANOVA with SPSS version 10.0. Homogeneous subsets were determined using Tukey's HSD.

DNA sequences were aligned and edited using CLUSTAL X v. 1.8 (Thompson and others 1997). Phylogenetic relationships were determined using PHYLIP v. 3.573 (Felsenstein 1985) programs for parsimony, maximum-likelihood, and distance methods. To determine the support for each clade, 1000 bootstrap replicates were used (100 in the maximum likelihood analysis). Phylogenetic trees were drawn using the TREEVIEW program (Page 1996).

### Results and Discussion\_\_\_\_\_

#### Morphology

Madrone isolates of *Nattrassia mangiferae* have hyaline, aseptate, guttulate conidia becoming 1- to 3-septate, verisicolored or brown (figure 3). Guttules were not visible in mature conidia. Conidia were thin-walled, and mostly fusoid in shape but occasionally oblong or clavate with truncate bases. The isolates differed in conidial length, width, and L:W, as well as in arthrospore size. Although there were statistically significant differences, there does not appear to be a geographical pattern in spore size or shape.



**Figure 3**—Arthrospores and macroconidia of *N. mangiferae* taken from a madrone canker.

*Fusicoccum* anamorphs of *Botryosphaeria* are described as having aseptate, hyaline conidia which may turn dark and septate with age. They are thin-walled and fusoid with truncate bases. A summary of conidium morphology and size is presented in table 1. *Nattrassia* is the only species in this group possessing arthrospores. *B. ribis*, its closest relative, has been reported to produce "chains of chlamydospore-like cells" (Rayachhetry and others 1996) or "swollen hyphal elements" (Morgan-Jones and White 1987) resembling arthrospores.

#### Phylogeny

ITS sequence analysis using all three methods placed the madrone isolates of *N. mangiferae* in the genus *Botryosphaeria* with *B. ribis* as its closest relative (figure 4). This dataset was not sufficient to resolve *B. dothidea* and *B. ribis* into strongly supported clades when *B. eucalyptorum* and *B. protearum* were added. However, other authors have

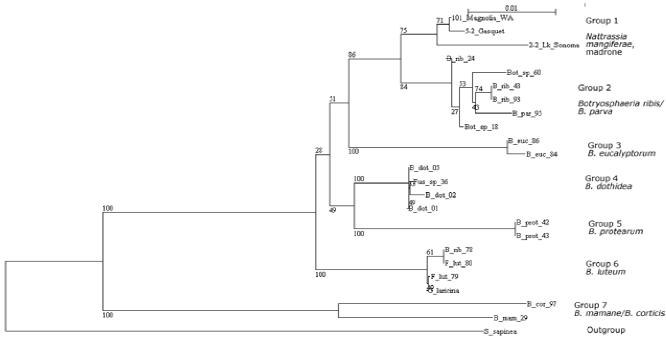
**Table 1**—Morphological characteristics of *Nattrassia mangiferae* and related species. ITS groups refer to Figure 4. Ranges of spore sizes are given in µm.

Species	s Ascospores			Conidia		
00000	Length Width Description			Length Width Description		
Nattrassia mangiferae (Sutton and Dyko 1989)	unknown	unknown	unknown	10–16 Arthrospores: 4–16.8	3.5–6.5 Arthrospores: 8.5	Hyaline, aseptate, fusoid, becoming 2–3 septate with central cell dark brown; L/W ratio= 0.6
<i>Exosporina fawcettii</i> (Wilson 1949)	unknown	unknown	unknown	11.9–16 Arthrospores: 4.6–7.3	3.2–6.5	Hyaline, aseptate, fusoid, becoming 2–3 septate with central cell dark brown;L/W ratio=2.7
Nattrassia mangiferae, Madrone isolates (ITS Group 2)	unknown	unknown	unknown	Macro: 15–25 Micro: 3-4 Arthrospores: 5-12.5	Macro: 5–7.5 Micro: 1 Arthrospores: 2.5-10	Macro: hyaline, aseptate, fusoid, becoming 2–3 septate with central cell dark brown; L/W ratio= 3.2; Micro: hyaline, oblong
Botryosphaeria ribis (Morgan-Jones and White, 1987, Rayachhetry and others, 1996) (ITS Group 1)	17–23	7–12	Hyaline, aseptate, ovoid	Macro: 17–25 Micro: 2–3 Chlamydo- spores: 18.6 (maximum)	Macro: 5.7 Micro: 1 Chlamydo- spores: 8.6 (maximum)	Macro: hyaline, fusoid, becoming 1-3 septate L/W ratio = 3.1;Micro: hyaline, allantoid
Botryosphaeria parva (Pennycook and Samuels 1985) (ITS Group 1)	18–27	8–11	Hyaline, aseptate becoming light brown and 1- or 2- septate.	15–20	4.5–6	Hyaline, aseptate, thin walled, rarely becoming darker and 1- septate, L/W ratio= 3.2
Botryosphaeria eucalyptorum (Smith and others, 2001) (ITS Group 3)	20–28	7–11	Hyaline, aseptate, granular, becoming light brown with age, fusoid	18–25	7–12	Hyaline, granular, ovoid to clavate, subtruncate base, aseptate; L/:W ratio= 2.2
Botryosphaeria dothidea (Crous and Palm 1999) (ITS Group 4)	18–25.5	7.5–12	Hyaline, aseptate, widest in the middle to upper third	21–28.5	4–4.5	Hyaline, aseptate, thin walled, rarely becoming darker and 1- septate; L/W ratio= 5.3
Botryosphaeria protearum (Denman and others 2003) (ITS Group 5)	25–37	9–13	Hyaline, aseptate, granular, becoming light brown with age, fusiform, inequilateral	Macro: 20–40 Micro: 3–6	Macro: 7–10 Micro: 1–1.5	Macro: hyaline, ovoid to clavate, granular, becoming irregularly fusiform, base bluntly rounded; L/W ratio= 3.5; Micro: smooth, aseptate, rod-shaped with rounded ends
Botryosphaeria luteum (Pennycook and Samuels 1985) (ITS Group 6)	18–22.5	7.5–12	Hyaline, aseptate	18–22.5	4.5–6	Hyaline, aseptate, thin walled; L/W ratio= 3.6

done this in datasets without these two closely related species (Jacobs and Rehner 1998, Smith and Stanosz 2001). There is some controversy over whether *B. ribis* and *B. dothidea* are different species or are both part of a single species complex. Isolates of *N. mangiferae* from Pacific

madrone clearly belong in this group and are more closely related to *B. ribis* than to *B. dothidea*.

There were two smaller clades within *Nattrassia* due to a single bp difference in the 5.8s region. This region is identical within *Botryosphaeria* (Zhou and Stanosz 2000).



**Figure 4**—Phylogenetic relations among *Nattrassia mangiferae* isolates from Pacific madrone and other *Botryosphaeria* and *Fusicoccum* species using the NJ (Neighbor Joining) method of Saitou and Nei (Thompson and others. 1997). The distance matrix was calculated using Kimura's 2-parameter model (Felsenstein 1989). Statistical support for branches was based on 1000 bootstrap replicates.

### Conclusions

*Nattrassia mangiferae* isolated from Pacific madrone belongs in the teleomorph genus *Botryosphaeria*. Disease cause by this fungus can be managed using methods developed for other *Botryosphaeria* pathogens, such as *B. dothidea*.

### Acknowledgements\_

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### **References\_**

- Crous, P. W. and M.E. Palm. 1999. Reassessment of the *Botryosphaeria* anamorph genera *Fusicoccum, Dothiorella*, and *Botryodiplodia*. Sydowia 51: 167–175.
- Denman, S., Crous, P. W., Groenewald, J. Z., Slippers, B., Wingfield, B., and M. J. Wingfield. 2003. Circumscription of *Botryosphaeria* species associated with Proteaceae based on morphology and DNA sequence data. Mycologia 95: 294–307.
- Felsenstein, J. 1989. PHYLIP—Phylogeny Inference Package (Version 3.2). Cladistics 5: 164–166.

- Jacobs, K. A. and S. A. Rehner. 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. Mycologia 90: 601–610.
- Morgan-Jones, G. and J. F. White. 1987. Notes on Coelomycetes, II. Concerning the *Fusicoccum* anamorph of *Botryosphaeria ribis*. Mycotaxon 30:117–125.
- Page, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357–358.
- Pennycook, S. R. and G. J. Samuels. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. Mycotaxon 24: 445–458.
- Rayachhetry, M. B., Blakeslee, G. M., Webb, R. S., and J. W. Kimbrough. 1996. Characteristics of the *Fusicoccum* anamorph of *Botryosphaeria ribis*, a potential biological control agent for *Melaleuca quinquenervia* in South Florida. Mycologia 88:239– 248.
- Smith, H., Crouse, P. W., Wingfield, M. J., Coutinho, T. A., and B. D. Wingfield. 2001. *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex in *Eucalyptus* in South Africa. Mycologia 93: 277–285.
- Smith, D. and G. R. Stanosz. 2001. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum anamorphs*. Mycologia 93: 505–515.
- Sutton, B. C., and B. J. Dyko. 1989. Revision of *Hendersonula*. Mycol. Res. 93:466–488.
- Thompson, J. D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 24:4876–4882.
- Wilson, E. E. 1949. The pycnidial stage of the walnut branch wilt fungus, *Exosporina fawcettii*. Phytopathology 53:705–712.
- Zhou, S. and G. R. Stanosz. 2000. Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8s rDNA sequences. Mycologia 93: 516–527.



### Could White Pine Blister Rust Spread by Atmospheric Transport from California to New Mexico?

#### Katrina L. Frank, Laurence S. Kalkstein, Brian W. Geils, Harold Thistle, Eugene P. Van Arsdel

Abstract—White pine blister rust (WPBR) was introduced into western North America near Vancouver, British Columbia in 1910. The rust spread thereafter in a series of jumps during favorable years. A description of the spatial and temporal patterns of long-distance WPBR spread is useful for assessing the risk of further spread in the Great Basin, Southern Rockies, and into Mexico and investigating possible gene flow among western populations. We group patterns of large-scale, upper-level atmospheric conditions and rank these groups for transport capacity with an Upper Level Synoptic Index (ULSI). We then select and rank periods when surface conditions are considered favorable for aeciospore deposition, germination, and infection. Circumstantial evidence supports the hypothesis that WPBR in the Sacramento Mountains is the result of a single introduction by long-distant atmospheric transport from the Sierra Nevada and that spread in this situation is restricted not by transport opportunities but infection requirements.

#### Introduction\_\_\_\_

White pine blister rust (WPBR) was introduced into western North America near Vancouver. British Columbia in 1910 (Mielke 1943). The rust spread thereafter in a series of jumps during favorable years. By 1942, rust distribution included much of the range of white pines in British Columbia, Washington, Oregon, northern Idaho, and northern California (Mielke 1943). Within a few more decades, the rust spread throughout the Sierra Nevada, the Canadian-Northern Rockies, and into the Central Rockies (see Smith and Hoffman 2000). Hawksworth (1990) first reported WPBR in the Sacramento Mountains of New Mexico, over a thousand kilometers from the nearest known infestations in California, Idaho, or Wyoming. Van Arsdel and others (1998) suggested the New Mexico infestation may have resulted from long-distance, atmospheric transport rather than human introduction. Hamelin and

others (2000) provided genetic evidence the New Mexico rust may have originated from California or other western locations.

A description of the spatial and temporal patterns of longdistance WPBR spread is useful for assessing the risk of further spread in the Great Basin, Southern Rockies, and into Mexico and investigating possible gene flow among western populations. In this study, we apply our understandings of rust epidemiology and meteorology to describe when and how often atmospheric conditions might be suitable for spread of WPBR from the Sierra Nevada and establishment in the Sacramento Mountains. We group patterns of large-scale, upper-level atmospheric conditions and rank these groups for transport capacity with an Upper Level Synoptic Index (ULSI). We then select and rank periods when surface conditions are considered favorable for aeciospore deposition, germination, and infection. Finally, we combine the upper level-transport index and the surface-infection index to determine the relative likelihood of successful rust spread from 1965 to 1974.

### Methods\_\_\_\_\_

The Upper Level Synoptic Index provides a ranking to identify periods when atmospheric conditions over western North America are suitable for long-distance transport of rust spores. Atmospheric data are based on historic weather records (UCAR 2003) processed by the NCEP/NCAR Reanalysis Project (NOAA-CIRES 2002). The processed data matrix consists of four meteorological variables, projected on a 6-hour frequency (observations) over a 2.5° latitude by 2.5° longitude grid. The variables are height of the 500 mb pressure surface (m), specific humidity (kg/kg), u-wind component (m/s) and v-wind component. Our study area extends from 20°N to 60°N and 60°W to 140°W; our study tracks the period 1965 to 1974.

The data were systematically sampled and subjected to principal components analysis. Retained components were entered into a clustering algorithm and 16 clusters identified. Clusters were characterized by the season of their most common appearance and their structure. Isopleths of pressure surface height were mapped for each cluster using a median observation time-point (date and hour). The mean 500 mb flow pattern for each cluster was

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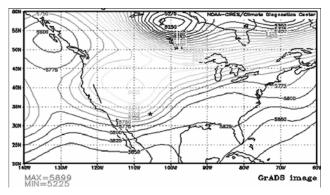
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examined and rated for the degree to which the flow passes over the Sierra Nevada and Sacramento Mountains. The Summer-trough pattern (figure 1) was rated very high; the Winter-trough-ridge and Summer-trough-ridge patterns were rated as high; the Winter-zonal-barotropic was rated moderate; and all other clusters were rated as low likelihood of transport. Persistence of favorable flow for transport was determined with a complex algorithm using an 18-hour moving average of transport ranks (1 to 4) with a penalty to reduce scores of nonconsecutive events. With this procedure, we determined relative likelihood of upper level flow events from 1965 to 1974 for proper orientation and duration to transport rust spores from the Sierra Nevada to the Sacramento Mountains.

The surface condition analysis ranked periods for concordance of atmospheric conditions over the Sacramento Mountains with epidemiological requirements for rust spores establish infection. For this analysis, we assumed establishment could occur only during April, May, June, or July. Other assumptions were that germination and infection requires a period of at least 6 hours of saturated air at the leaf surface and a temperature above 13° C within 3 weeks of spore deposition. Our estimates of surface conditions were from the NCAR reanalysis data set and used the grid point nearest to the Sacramento Mountains. We assumed that saturated air at the leaf surface would correspond in this regional data set as a relative humidity greater than 85 percent. We used a double weighting algorithm to score periods of suitable humidity and temperature for duration and proximity to the transport event and rank periods from their position in the distribution of scores. We, thereby, determined relative likelihood of surface condition periods throughout 1965-1974 when season, humidity, and temperature in the Sacramento Mountains would allow for rust infection.



**Figure 1**—An upper level synoptic pattern for a Summertrough over North America; also shown are the general location from which rust spores may originate(straight line) and the Sacramento Mountains, New Mexico (star). Air-flow and therefore spore transports tends to follow along pressure isobars (curved lines).

An infestation could only establish when atmospheric conditions permit both transport and infection. We summed the rank scores of transport and infection for each 6-hour observation and determined classification thresholds from a sensitivity analysis. With the combined score, we identified for each observation the relative likelihood of rust spread—transport and infection—as very high, high, moderate, or low.

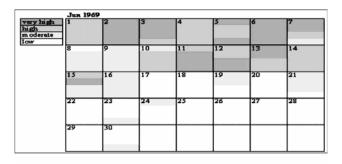
#### Results and Discussion\_

Atmospheric conditions have developed and persisted numerous times from 1965 to 1974 that satisfy expected requirements for the transport of WPBR aeciospores from the Sierra Nevada to Sacramento Mountains and their subsequent infection.

A not-unlikely potential for atmospheric transport from the Sierra Nevada to Sacramento Mountains occurs when any one of four synoptic patterns develop. Upper level air flow from west to east, from California to New Mexico is a common situation. Flow from the north-northwest or north that could transport rust spores from Idaho or Wyoming to New Mexico, however, is very uncommon, occurring in only seven percent of observations.

A synthesis of meteorology and epidemiology allows us describe in detail when and how often atmospheric conditions are suitable over sufficient duration for transport followed by infection to occur. Although our rankings for transport, for infection, and their linkage are relative, they serve as a useful filter to sort among the thousands of observations and identify the most relevant periods when rust spread could occur.

For the study period seasons, May through July from 1965 to 1974, we identified 33 observations (0.68 percent of all observations) with a very high score for rust spread. These 33 observations were nested into five periods of various durations in four of the ten years examined. In 1972, there was a single observation; in 1971, there were two nonconsecutive observations; in 1968, there were seven observations during the first week of July; and in 1969, there were 23 observations during the first two weeks of June. The single, extended period in early June, 1969 included three continuous periods of 24 to 42 hours when conditions for spread remained very high (figure 2). A successful establishment of WPBR in 1969 would be consistent with an estimate by Van Arsdel and others (1998) that the oldest cankers discovered in the Sacramento Mountains originated from infections occurring about 1970.



**Figure 2**—Calendar for June 1969, with periods indicated when conditions were very high, high, moderate, or low for long-distance spread and infection.

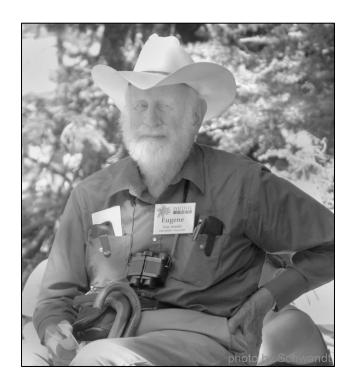
#### Conclusions and Implications

Direct observations of WPBR cankers date the rust as present in numerous locations of the Sierra Nevada after 1960 and in several locations of the Sacramento Mountains only ten years later. We have not yet found WPBR in Arizona or western New Mexico along the likely transport pathways. Hamelin and others (2000) found no genetic diversity within the Sacramento rust population. In this study, we found that upper level atmospheric conditions capable of transporting spores from the Sierra Nevada to the Sacramento Mountains are common (frequent each year), but only occasionally (at least once each decade) followed by surface conditions of proximity and duration suitable for infection.

Circumstantial evidence supports the hypothesis that WPBR in the Sacramento Mountains is the result of a single introduction by long-distant atmospheric transport from the Sierra Nevada and that spread in this situation is restricted not by transport opportunities but infection requirements. Although this is a single-case study, the synoptic methodology is readily applicable to other sites with and without current WPBR infestations. With further development, a relative hazard map of the southwestern US and northern Mexico could be produced.

#### References

- Hamelin, R.C., R.S. Hunt, B.W. Geils, G.D. Jensin, V. Jacobi, and N. Lecours. 2000. Barrier to gene flow between eastern and western populations of *Cronartium ribicola* in North America. Phytopathology. 90:1073–1078.
- Hawksworth, F.G. 1990. White pine blister rust in New Mexico. Plant Disease. 74:938.
- Mielke, J.L. 1943. White Pine Blister Rust in North America. New Haven, Conn.: Yale University Press.
- NOAA-CIRES Climate Diagnostics Center. 2002. http://www. cdc.noaa.gov/cdc/data.ncep.reanalysis.html. Accessed March 12, 2002.
- Smith, J.P., and J.T. Hoffman. 2000. Status of white pine blister rust in the Intermountain West. Western North American Naturalist. 60:165–179.
- UCAR. April 1, 2003. NCEP/NCAR Reanalysis Project Description.http://dss.ucar.edu/pub/reanalysis/rean\_proj\_des.html. Accessed August 4, 2003.
- Van Arsdel, E.P., D. A. Conklin, J.B. Popp, and B.W. Geils. 1998. The distribution of white pine blister rust in the Sacramento Mountains of New Mexico. p. 275–283 In: Proceedings of the First IUFRO Rusts of Forest Trees Working Party Conference. Saanelka, Finland: Finnish Forest Research Institute.



#### Lodgepole pine dwarf mistletoe, a little different.

Arceuthobium americanum is a common, widespread, and damaging mistletoe principally on lodgepole pine (*Pinus contorta*) but occasionally or rarely on several conifer species that occur within the Jefferson region whitebark, Jeffery, and ponderosa pines and Douglas-fir. The shoots are a typical mistletoe height of 5 to 9 cm, but a little finer at 1–3 mm than many other mistletoe species. The most distinctive character (for mistletoes of Canada and the United States) is the verticillate branching pattern.

#### Mistletoe identification

The reduction and simplification of mistletoe morphology due to its parasitic habit and similarity due to its close phylogeny do make species identification difficult. Taxonomy and systematics are also controversial and therefore subject to different interpretation. Except in challenges such as a quiz to "Name that mistletoe", we are interested in populations of plants in natural communities with known associations and locations. Host preferences, phenology, and disease reactions (brooming) are as informative as shoot and flower morphology (color, size, proportions, and branching pattern). Keys, text descriptions, and even pictures have some but limited value. Experience with the variety of populations and comparison to reference collections are proper. Generally, if you know the host, where you are, and what are the likely mistletoes, identification is possible.



#### A picture guide

Although we provided keys and descriptions in the Mistletoes of North American Conifers, cost and availability prohibited including a complete galley of detailed, color images of each mistletoe. Color photographs of each dwarf mistletoe were presented in the Hawksworth and Wiens monograph (Agriculture Handbook 709); these published and online images, however, were scanned from 35 mm slides of various quality.

A galley of high-quality, color portraits of each mistletoes species would provide a useful supplement to the Mistletoes of North American Conifers. I (Brian Geils) am now compiling and archiving such a gallery. I will accept and credit digital images in JPEG-format (with date, location, host, and identification data) from contributors. Each species would be represented by close portraits for staminate flowering, pistillate flowering and fruiting plants. Those judged best for illustrating the species taxonomically and aesthetically would be included in an online and printed publication.



## Genetic Variation of *Armillaria* ostoyae within the Western United States.

### J.W. Hanna, N.B. Klopfenstein, M.-S. Kim, G.I. McDonald, and J.A. Moore

Abstract-Intraspecific and intragenomic variation of Armillaria ostoyae were observed through sequencing of ribosomal DNA (rDNA) including nuclear large ribosomal subunit (nLSU), internal transcribed spacer (ITS), 5.8S rDNA, and intergenic spacer (IGS-1). Many of the A. ostoyae genets contained heterogeneous sequences, an indication of intragenomic variation/intraspecific hybridization. Intragenomic variation was verified by visual analysis of sequence chromatograms and PCR with specific internal primers. Intraspecific and intragenomic variation was found to exist in all rDNA regions analyzed, with the exception of the 5.8S rDNA. Variation will be further analyzed using Parsimony and Neighbor-Joining methods for phylogenetic analysis. Genetic diversity within A. ostoyae can be examined for relationships to ecological function (for example, pathogenicity, host specificity, and habitat type), geographic origin, forest management practices (for example, fertilization), and interactions among Armillaria genotypes.

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# First Report of A1 Mating Type of *Phytophthora ramorum* in North America

Everett M. Hansen, Paul W. Reeser, Wendy Sutton, Loretta M. Winton, Nancy K. Osterbauer

#### Introduction\_

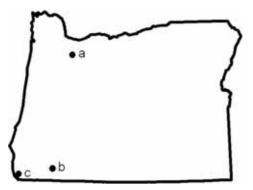
Phytophthora ramorum is known from Europe and the West Coast of the United States (Davidson 2003). In Europe, it is found in nurseries and landscape plantings. In the United States, it has been confined to coastal forests and, in California, a few horticultural nurseries. All European isolates tested have been A1 mating type, while all Oregon and California isolates were A2 mating type (Werres 2001). AFLP markers also indicated that the populations on the two continents are genetically distinct and that nearly all North American isolates are from a single clone (Ivors 2002). In June 2003, P. ramorum was isolated from diseased Viburnum (figure 1), Camellia, and Pieris cultivars from two Oregon horticultural nurseries, one in Clackamas County and another in Jackson County (figure 2). As part of the effort to determine the origin of these infestations, we tested the nursery isolates for mating type and compared their genotypes with those of known European and Oregon forest isolates using DNA microsatellite markers.



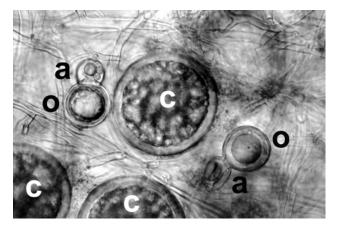
Figure 1—Infected Viburnum in nursery production block.

#### Mating Types

Mating type was determined by pairing seven Oregon nursery isolates, three Oregon forest isolates (representative of the predominant North American clone), and two European nursery isolates. Agar plugs from 3-day old colonies were placed in close proximity on carrot agar plates. Plates were examined for oogonia (figure 3) after 3 days and 10 days (Brasier unpublished). Genotype was determined using four polymorphic microsatellite loci (figure 4) that distinguish *P. ramorum* isolates from Europe and North America.



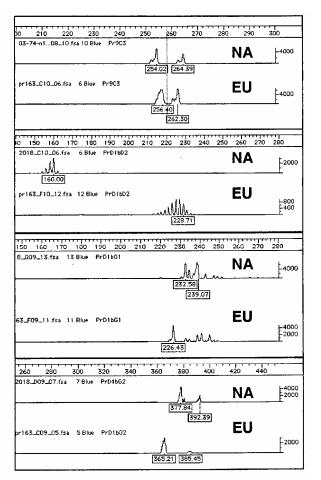
**Figure 2**—Locations in Oregon where *Phytophthora ramorum* has been isolated. a—nursery in Clackamas Co., b—nursery in Jackson Co., c—forest near Brookings.



**Figure 3**—Oospores (o) with amphigynous antheridia (a) generated by paired A1 and A2 isolates of *P. ramorum*. Large spherical structures are chlamydospores (c).

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**Figure 4**—Allele sizes of North American (NA) and European (EU) *P. ramorum* isolates at four polymorphic microsatellite loci.

Oogonia and antheridia typical of *P. ramorum* formed when isolates from Clackamas County were paired with the Oregon forest isolates and when Jackson County isolates were paired with the European isolates. Sexual structures also formed in pairings between Oregon forest isolates and European isolates, but not in any other combinations. Microsatellite marker patterns of Clackamas County isolates were identical to the European isolates. Marker patterns of the Jackson County isolates were identical to the Oregon forest isolates (table 1).

Table 1—Mating type and microsatallite results.				
Isolate	Micro-	Mating	Host	Isolate
	satellite	Туре		Source
03-74-1	EU	A1	Pieris sp.	Clackamas
03-74-2	EU	A1	Viburnum sp.	Clackamas
03-116-1	EU	A1	Viburnum sp.	Clackamas
03-147-9	EU	A1	Pieris sp.	Clackamas
DO3-29B-1	EU	A1	Viburnum sp.	Clackamas
DO3-29B-2	EU	A1	Viburnum sp.	Clackamas
03-156-10B	NA	A2	Camellia sp.	Jackson
03-156-10A	NA	A2	Camellia sp.	Jackson
03-156-6	NA	A2	<i>Camellia</i> sp.	Jackson

These results indicate that the recent Oregon nursery infestations are of different origin. The Clackamas County isolates are of A1 mating type and European genotype. According to shipping records, the nursery has received no host nursery stock directly from Europe. However, host nursery stock has been received from a nursery in British Columbia, suggesting this may be the source of this infestation. The Jackson County isolates are of A2 mating type with a microsatellite pattern similar to the Oregon forest isolates. The latter result is consistent with the reported origin of these infested plants from a California nursery (CDFA, personal communication). The Oregon nursery infestations highlight the dangers of unregulated or under-regulated transport of host nursery stock from infested areas to non-infested areas. All host plants from infested nursery blocks at the affected Oregon nurseries have been destroyed by incineration (figure 5) and a regular monitoring program implemented. Other host nursery stock on site have been taken off-sale pending verification of free-from disease status per USDA, Animal and Plant Health Inspection Service requirements.



Figure 5—Incineration of all plants in an infested nursery block

#### **References**

- Davidson, J. M., Werres, S., Garbelotto, M., Hansen, E. M., and Rizzo, D. M. 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. Online. Plant Health. http://www.plantmanagementnetwork.org/pub/php/diagnosticgui de/2003/sod
- Ivors K., Hayden, K., Garbelotto, and M., Rizzo, D. 2002. Molecular Population Analyses of *Phytophthora ramorum*. Sudden Oak Death Science Symposium, 15–18 December 2002, Monterey, California. Online: http://danr.ucop.edu/ihrmp/sod symp/paper/paper17.html
- Werres S., R. Marwitz, W.A. Man in 't Veld, A.W. De Cock, P.J.M. Bonants, M. De Weerdt, K. Themann, E. Ilieva, and R.P. Baayen, 2001. *Phytophthora ramorum* sp. nov: a new pathogen on *Rhododendron* and *Viburnum*. Mycological Research, 105 (10): 1155–1165.



## Is the Alternate Host for White Pine Blister Rust Present in Colorado?

Holly S. J. Kearns, William R. Jacobi, Kelly Sullivan, and Brian W. Geils

#### Introduction\_

White pine blister rust (*Cronartium ribicola*) is a disease of five needle pines. The disease recently spread into northern Colorado and threatens limber and Rocky Mountain bristlecone pine stands in the rest of the state. To determine if the alternate host (species of *Ribes*) is present near white pines in Colorado a survey was conducted in the summer of 2003.

#### Methods\_\_\_

A survey for the occurrence of *Ribes* species was conducted on four National Forests in Colorado (Pike, Rio Grande, Roosevelt, and San Isabel). This survey consisted of randomly located linear plots (20 ft by 200 ft) over a range of elevations, aspects, and habitat types on which the number of *Ribes* bushes, linear length of stems, and number of stems per bush by species were collected. On the four forests, 244 plots were established.

#### Results and Discussion\_\_\_\_\_

Five species of *Ribes* susceptible to white pine blister rust were found in the four National Forests surveyed within elevation ranges that correspond to the distribution of both limber and Rocky Mountain bristlecone pines (figures 1 to 5). Information was obtained on *Ribes* density, constancy, and elevation range (table 1 to 5). From these data it does not appear that the distribution of white pine blister rust within the sampled Colorado National Forests will be limited by the distribution of the alternate host. Future work will relate these data to developing a hazard model for white pine blister rust in the Central Rocky Mountains.



Figure 1—*Ribes cereum.* This currant is unarmed, has pink flowers, and red fruit.

 Table 1—Distribution of Ribes cereum in four Colorado

 National Forests.

Forest	Density <sup>a</sup>	Constancy <sup>b</sup>	Elevation <sup>c</sup>
Pike	784 (2570)	0.18 (13/72)	9390–10600
Rio Grande	1241 (4184)	0.17 (11/62)	8790–11200
Roosevelt	1591 (3616)	0.49 (29/59)	7770–9005
San Isabel	2632 (9075)	0.21 (11/51)	8400-10065
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<sup>a</sup>Mean ft of stem per acre (standard deviation). <sup>b</sup>Fraction of plots occupied (plots occupied/examined). <sup>c</sup>Elevation range, ft as lowest–highest observed.



Figure 2—*Ribes inerme.* This gooseberry usually has nodal spines and develops smooth red berry. The spots on this specimen are infections of *Cronartium ribicola.* Table 2—Distribution of *Ribes inerme* in four Colorado National Forests.

Forest	Density <sup>a</sup>	Constancy <sup>b</sup>	Elevation <sup>c</sup>	
Pike	3498 (10283)	0.34 (25/72)	9370–11800	
Rio Grande	2967 (11512)	0.27 (17/62)	8670–11200	
Roosevelt	1583 (2573)	0.61 (36/59)	7400-9005	
San Isabel	2632 (3650)	0.43 (22/51)	8400–11501	
and the test			<b>`</b>	

<sup>a</sup>Mean ft of stem per acre (standard deviation).

<sup>b</sup>Fraction of plots occupied (plots occupied/examined).

<sup>c</sup>Elevation range, ft as lowest–highest observed.

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**Figure 3**—*Ribes laxiflorum.* This currant is distinguished by a creeping habit.

**Table 3**—Distribution of *Ribes laxiflorum* in four Colorado National Forests.

Forest	Density <sup>a</sup>	Constancy <sup>b</sup>	Elevation <sup>c</sup>
Pike	17 (135)	0.02 (2/72)	10700–10925
Rio Grande	109 (778)	0.03 (2/62)	9730–9970
Roosevelt	0	- (0/59)	_
San Isabel	0	- (0/51)	_

<sup>a</sup>Mean ft of stem per acre (standard deviation). <sup>b</sup>Fraction of plots occupied (plots occupied/examined). <sup>c</sup>Elevation range, ft as lowest–highest observed.



**Figure 4**—*Ribes lacustre.* This spiny currant has flowers similar to those of *R. montigenum*, but the glandular fruit develops a deep blue color and leaves are not hairy. (photo by M. Newcomb)

**Table 4**—Distribution of *Ribes lacustre* in four Colorado National Forests.

Forest	Density <sup>a</sup>	Constancy <sup>b</sup>	Elevation <sup>c</sup>	
Pike	0	- (0/72)	-	
Rio Grande	0	- (0/62)	-	
Roosevelt	285 (1247)	0.10 (6/59)	8540–9390	
San Isabel	0	- (0/51)	_	
a				

<sup>a</sup>Mean ft of stem per acre (standard deviation). <sup>b</sup>Fraction of plots occupied (plots occupied/examined). <sup>c</sup>Elevation range, ft as lowest-highest observed.



**Figure 5**—*Ribes montigenum.* This other spiny currant develops bright red, glandular fruits and usually has hairy leaves.

Table 5—Distribution of Ribes montigenum in four Colorado
National Forests.

Forest	Density <sup>a</sup>	Constancy <sup>b</sup>	Elevation <sup>c</sup>	
Pike	3183 (9784)	0.37 (27/72)	10000-12000	
Rio Grande	6703 (14926)	0.48 (30/62)	9730–11840	
Roosevelt	0	- (0/59)	-	
San Isabel	1187 (3255)	0.17 (9/51)	10500-11800	

<sup>a</sup>Mean ft of stem per acre (standard deviation). <sup>b</sup>Fraction of plots occupied (plots occupied/examined). <sup>c</sup>Elevation range, ft as lowest–highest observed.

