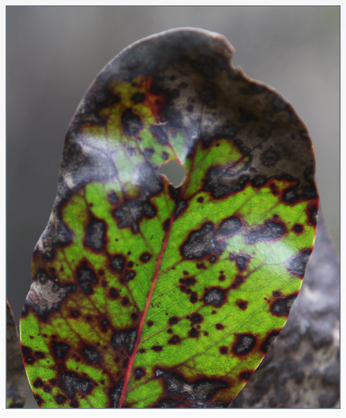
Identification of *Phacidiopycnis washingtonensis*—a fungus associated with madrone leaf blight

Leaf blight—consisting of browned, desiccated leaves occurring mainly in the lower parts of the canopy—has been reported on Pacific madrone throughout its range and generally occurs during periods of wet spring weather. In May 2009 and 2011 severe outbreaks occurred and leaves from madrones growing in Washington and Oregon were sampled to determine the causal organism.

Symptomatic leaves were collected from each of 23 madrone trees at WSU-Puyallup, in May 2009. Segments of symptomatic tissue about 25 mm square were cut from the leaves and surface-sterilized in 10% sodium hypochlorite, rinsed in sterile deionized water, blotted dry, and cultured on 2% malt extract agar (MEA).

Culture of Phacidiopycnis washingtonensis *on PDA.*

Fifty percent of the leaf blotch and 30% of leaf spot samples contained a fungus that was fast-growing (20 mm diameter in four days) and produced colonies that were a pale grey with dark grey reverse and a felty texture. Pycnidia formed which exuded conidia in peach-colored droplets after one week at ambient temperatures with alternating light and dark intervals. This fungus was isolated onto MEA and the ITS region was sequenced after PCR using universal primers (1). A BLAST search showed 100% similarity to *Phacidiopycnis washingtonensis* (GenBank accessions JQ743784–JQ743786). This fungus is reported to cause rot on apple fruits in cold storage as well as canker and twig dieback of apple and pear trees (2).



Two types of symptoms were observed on *A. menziesii*. These were leaf necrosis or blotching along the edges of the leaves and at the tips, and leaf spots (*left*).

The fungus was also isolated from leaf spots on emerging foliage, lesions on green shoots, and the petiole and leaf blade of dead, attached leaves (*below*).

Visit the WSU Pacific Madrone Research Website at http://www.puyallup.wsu.edu/ppo/madrone/

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A NEW LEAF BLIGHT DISEASE ON PACIFIC MADRONE (*ARBUTUS MENZIESII*) CAUSED BY *PHACIDIOPYCNIS WASHINGTONENSIS* IN WESTERN WASHINGTON AND OREGON

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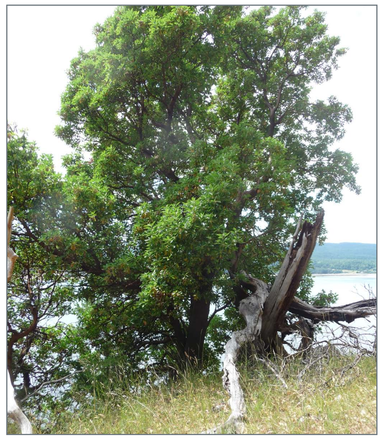
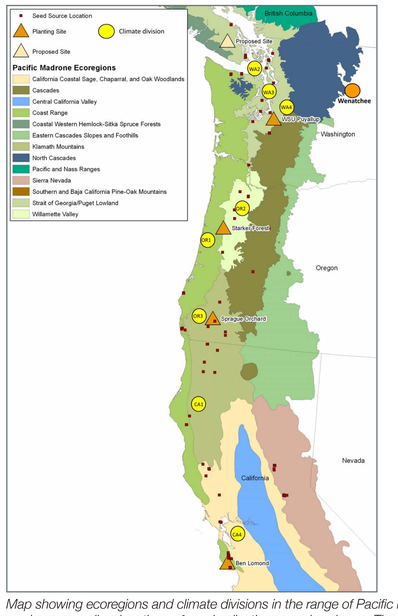
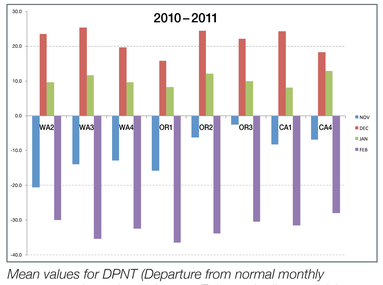
Phacidiopycnis washingtonensis is a newly recognized foliar pathogen associated with foliar blight on madrone and is known as a cold temperature pathogen on other hosts such as apple (*Malus* spp.), pear (*Pyrus communis*), and persimmon (*Diospyros kaki*). It has also been isolated from strawberry tree (Arbutus unedo), *Rhododendron* spp. and kinnikinnick (*Arctostaphylos uva-ursi*). Damage from foliar blight attributed to *P. washingtonensis* was especially severe in 2010–2011, which was a strong La Niña year with periods of extreme cold temperatures followed by extended periods of cool, wet weather in the Pacific Northwest.



Healthy (left) and blighted (above) madrone trees. The disease is not normally fatal unless the terminal buds are killed and the tree fails to produce new foliage in the summer. Conditions of high humidity and cold temperatures favor disease development.



Map showing ecoregions and climate divisions in the range of Pacific madrone. Sites of common gardens for a genetic study of madrone as well as locations of seed collections are also shown. The common garden sites will be monitored for various diseases, including leaf blight, and resistant trees will be identified.



Mean values for DPNT (Departure from normal monthly temperature in tenths of degrees F) for each climate division, for winter 2010-2011. Data from NOAA (www7.ncdc.noaa.gov/CDO/cdo).

Climate Divisions

WA2	*Northeast Olympics, San Juan Islands*
WA3	*Puget Sound Lowlands*
WA4	*East Olympics, Cascade Foothills*
OR1	*Coastal Area*
OR2	*Willamette Valley*
OR3	*Southwestern Valleys*
CA1	*North Coast Drainage*
CA4	*Central Coast Drainage*

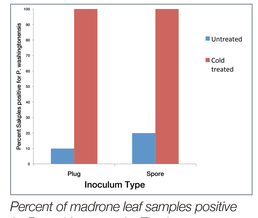
Pathogenicity of an apple isolate of *P. washingtonensis* on Pacific madrone and the effects of cold treatment on lesion development

To determine whether apple isolates of *P. washingtonensis* cause leaf blight on Pacific madrone, greenhouse-grown madrone seedlings were inoculated with an isolate of the fungus collected from Red Delicious apple. Prior to inoculation, one set of leaves were predisposed to cold injury with a commercial aerosol Freeze-IT (–51°C) (Curtin Matheson Scientific, Houston, TX), held at a distance of 2–3 cm for 10 seconds and allowed to dry for 10 minutes. Inoculum was applied to 1 cm^2 marked areas of cold-treated and untreated foliage using two methods. A conidial spore suspension at a concentration of 5×10^5 conidia/ml was sprayed on the upper surface of one group, and a mycelial plug from a PDA culture of the fungus was applied to the upper surface of the second group. Leaves were enclosed in plastic bags to retain moisture and monitored for symptoms for four weeks. Controls for each treatment were sterile water spray and PDA plugs.

Cold-treated (left) and untreated (right) madrone leaves inoculated with a spore suspension of P. washingtonensis *showing symptoms on the cold-treated leaf.*

Cold-treated (left) and untreated (right) madrone leaves inoculated with a mycelial plug of P. washingtonensis. *As with the spore inoculum, the cold-treated leaf has blight symptoms.*

All inoculated cold treated leaves showed clear symptoms of leaf blight four weeks after inoculation, and no symptoms appeared on non-cold treated leaves. Untreated leaves were left in the greenhouse for another two weeks to check for appearance of symptoms. Leaf segments from marked areas of symptomatic and asymptomatic leaves were surface-sterilized and placed on APDA. Samples were incubated at 20°C for five days in dark. *P. washingtonensis* was isolated successfully from 100% of all the cold treated leaves with both types of inoculum, and no *P. washingtonensis* was isolated from non-cold treated leaves. Further isolation from non-cold treated leaves was done without surface sterilization to validate if the pathogen survived on the surface of the leaves. The pathogen was re-isolated from 20% of leaves inoculated with fungal spores and 10% of leaves inoculated by fungal mycelium. While cold-treated, non-inoculated leaves had some symptoms of damage, no pathogen was isolated from non-inoculated control leaves, both with and without the cold treatment.



Percent of madrone leaf samples positive for P. washingtonensis. *The fungus was consistently isolated from cold-treated leaves. These samples were not surface-sterilized, showing that the fungus was surviving on the leaf surface but not colonizing the tissue.*

References

1. *White, T. J., Bruns, T., Lee, S., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal DNA for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, New York, NY.*
2. *Xiao, C.L., Rogers, J.D., Kim, Y.K., and Liu, Q. 2005.* Phacidiopycnis washingtonensis *– a new species associated with pome fruits from Washington State. Mycologia 97: 464-473.*

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