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).

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 a pale grey with dark grey reverse and a felty texture. 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 accession 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).

Phacidiopycnis washingtonensis is a newlyrecognized 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-ursa). 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.

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 cm2 marked areas of cold-treated and untreated foliage using two methods. A conidial spore suspension at a concentration of 5 × 106 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.

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 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 with 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.

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

Ph. romanum A is a newly recognized foliar pathogen associated with foliar blight on madrone. The disease is primarily leaf discoloration in populations with the same symptoms as the winter moth. The fungus was also isolated from 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 cm2 marked areas of cold-treated and untreated foliage using two methods. A conidial spore suspension at a concentration of 5 × 106 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.