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First Report of *Phytophthora ramorum* Infecting Mistletoe in California

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Since 2005, our research on the spread and development of *Phytophthora ramorum* on grand fir (*Abies grandis*) and Douglas-fir (*Pseudotsuga menziesii*) at a Christmas tree plantation near Los Gatos, CA, has shown that distance from infected plants, predominantly California bay laurel (*Umbellularia californica*), is an important factor relating to the infection of these two conifer species (1).

In 2005 and 2006, a few white fir (*Abies concolor*) and Douglas-fir Christmas trees with a limited number of *P. ramorum*-infected shoots were found at another farm near our research site. This was unexpected because no California bay laurel or any other known hosts of *P. ramorum* were in close proximity. The trees were located beneath a large black walnut tree (*Juglans nigra*) that was infected with the hemiparasitic plant, mistletoe (*Phoradendron serotinum* subsp. *macrophyllum*).

In May 2006, mistletoe leaves, stems, and inflorescences with dark spots that had fallen to the ground or onto Douglas or white fir branches from the walnut tree described above were collected. Walnut catkins lying on top of newly infected Douglas-fir shoots and fresh walnut leaves with dark spots, from shoots growing at the base of the tree, were also collected. Tissues were plated on corn meal agar amended with ampicillin, rifampicin, and pimaricin (CARP) medium. Although no *P. ramorum* was recovered from any of the walnut material, mistletoe leaf, or stem tissue, it was isolated from a blackened mistletoe inflorescence (Fig. 1). The isolate was genetically characterized at eight microsatellite loci: Pr9C3, PrMS6, and PrMS9 (2); PrMS39, PrMS43, and PrMS45 (3); and 18 and 64 (4), and determined to be of the NA1 lineage.

In 2007, Koch's postulates were completed with the inoculation and subsequent reisolation of *P. ramorum* from mistletoe. Fresh, uninfected plant material was collected from California in February and transported to an APHIS-approved facility in Puyallup, WA. Because mistletoe is a hemiparasite that depends on the xylem of its host for water, it was not feasible to test its host status *in situ*. In order to keep the study material as close to natural as possible, branches harvested from mistletoe plants were inserted through Parafilm (Pechiney Plastic Packaging Co., Chicago, IL) into 50-ml flasks filled with deionized water. The isolate from the 2006 collection was grown on 1/3 V8 agar medium, and ~2 ml of a 1.7×10^5 -zoospores/ml suspension was misted on each of eight branches using an airbrush sprayer. Eight control branches were sprayed with water. The flasks were placed in opaque plastic tubs with ~2.5 cm of warm water (37°C) to provide humidity, covered with another



Fig. 1. Blackened mistletoe inflorescence stalk (arrow) collected from Christmas tree site.

tub, and stored in a biocontainment unit with 24-h light. The air temperature within the tubs ranged from 17°C to 24°C for 2.5 h before stabilizing at the storage temperature of 15°C to 16°C.

After 9 days, symptoms on the eight inoculated branches included brown, black, or gray spots on leaves and inflorescence stalks (Fig 2), black lesions on stems (Fig. 3), and a browning of normally white berries. Six control branches also had symptomatic leaves, stems, and berries. Isolations from symptomatic tissue were surface sterilized with a 0.6% sodium hypochlorite solution and plated on CARP. The eight inoculated branches yielded *P. ramorum* from leaves, stems, and inflorescence stalks; a single berry from one of the branches also yielded the pathogen. Isolations from the six symptomatic control branches yielded no *P. ramorum*.



Fig. 2. Symptomatic leaves and inflorescence of mistletoe 9 days after inoculation with *P. ramorum*.



Fig. 3. Symptomatic mistletoe stem 9 days after inoculation.

Our results indicate that a hemiparasite, mistletoe, can serve as a host of *P. ramorum*. These plants are collected and distributed for holiday decorations, but it is unknown if this activity contributes to the spread of the pathogen.

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