

New Relationships Among the Sudden Oak Death Pathogen, Bark and Ambrosia Beetles, and Fungi Colonizing Coast Live Oaks¹

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Abstract

Sudden oak death (SOD) has had devastating effects on several oak species in many California coastal forests. *Phytophthora ramorum* has been identified as the primary causal agent of sudden oak death. While the pathogen may be capable of killing mature trees, it is likely that in nature opportunistic organisms play significant roles in the decline and death of infected trees. For example, we have found elevated landing rates of bark and ambrosia beetles (Coleoptera: Scolytidae) on mechanically inoculated coast live oaks (*Quercus agrifolia*) in California. The tunneling activity of these beetles in bleeding cankers on *P. ramorum*-infected coast live oaks may accelerate mortality and may contribute to catastrophic failures, even while diseased trees retain asymptomatic canopies. The objective of this study was to determine the role of bark and ambrosia beetle infestation in the introduction and/or stimulation of decay fungi associated with tree mortality and breakage. We inoculated coast live oaks with *P. ramorum* in two forested sites in Marin County in March 2005 and monitored them for signs and symptoms of *P. ramorum* infection. An additional group of asymptomatic trees was felled to allow colonization by bark and ambrosia beetles. In January and July of 2006, we randomly selected and harvested three *P. ramorum*-inoculated trees and three asymptomatic trees from each of the sites. Trees selected for fungal culturing were in the following categories: (1) Asymptomatic trees; (2) Live symptomatic trees exhibiting only bleeding without obvious beetle attacks; (3) Live symptomatic trees exhibiting bleeding with beetle attacks; (4) Dead symptomatic trees with beetle attacks; (5) Dead asymptomatic trees without beetle attacks; (6) Dead asymptomatic trees with beetle attacks. Trees were cut a minimum of 30 cm below the point of inoculation, generating bolts approximately 70 cm long. Each bolt was cut into 15 cm thick disks. Wood samples (5 to 10 mm wide, four per disk) were collected along cross-sectional transects from the upper surface of each disk and divided into four sections. Each section was placed on one of several types of media: potato dextrose agar, malt extract agar and water agar. We separated and purified morphologically distinct fungal colonies (morphotypes) and amplified the internal transcribed spacer (ITS) region of the rDNA operon. Amplicons were sequenced and blasted in GenBank (<http://www.ncbi.nlm.nih.gov>). The principal taxa isolated from wood samples are described below.

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Pezicula cinnamomea was only isolated from bleeding trees before beetles attacked. Two species, *Botryosphaeria sarmentorum* and an unnamed *Ascomycete* sp. were isolated from infected trees that had not been attacked by beetles, and also from trees that died following beetle infestation. The greatest numbers of fungi were isolated from beetle-colonized, living trees: *Botryosphaeria corticola*, *Geosmithia fassatia*, *Mucor racemosus*, *Trametes versicolor* and *Truncatella angustata*. Fungi were not isolated from the symptomatic dead trees that died before they had been colonized by beetles. Beetle-attacked, asymptomatic dead trees yielded *B. corticola*, a *Monochaetia* sp. and an *Alternaria* sp. All the fungi identified had ITS values of 97 percent or higher.

Pezicula cinnamomea is generally known as a pathogenic fungus that primarily causes dieback disease of *Quercus* spp., callus rings in *Fagus sylvatica* in Europe and has also been found in *Prunus avium* in Europe and in *Prunus* sp. in Japan. *Botryosphaeria corticola* causes cankers and dieback in *Quercus* spp., and *B. sarmentorum* has been associated with dieback and canker diseases of *Quercus* spp. and *Ulmus*, *Malus*, *Prunus*, and *Pyrus* spp. in Europe. *Geosmithia fassatia* is an anamorphic fungus found in association with scolytid bark beetle-colonized *Quercus pubescens* in central Europe. *Mucor racemosus* is a filamentous fungus found in soil, plants, decaying fruits and vegetables, while *Trametes versicolor* (known as the Turkey Tail fungus) is found ubiquitously in temperate to sub-tropical forests throughout the world where it serves as a primary decomposer of hardwoods, including *Quercus* spp. *Truncatella angustata* is known to cause disease on stems of *Ribes*, *Prunus* and *Malus* in England. The unknown ascomycete sp. has been isolated from Scots pine (*Pinus sylvestris*) sapwood, at the root collar or in roots. *Monochaetia* spp. cause cankers on several hardwood tree species. *Alternaria* spp. cause serious twig diseases on several hardwood trees, including apples.

These experiments will be repeated in 2007. Our work has revealed the presence of several fungal species commonly associated with disease and decay of hardwood species and appears to be a promising approach in our attempts to fully characterize fungal communities associated with the SOD syndrome. The greatest species diversity was found in infected trees after bark and ambrosia beetles had colonized the sapwood. This study will be expanded and refined so that we can determine the sequence of microorganisms that occur in oaks following infection with *P. ramorum*.

Key words: *Phytophthora ramorum*, sudden oak death, bark and ambrosia beetles, decay fungi, coast live oak.