The Potential of Ambrosia Beetles (*Monarthrum scutellar*e and *Monarthrum dentiger)* to Vector the Phytophthora that causes Sudden Oak Death

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Abstract Potential vectoring of invasive fungus, genus *Phytophthora,* in infected California oak trees by ambrosia beetles, *Monarthrum scutellare,* was observed. A newly discovered invasive fungus in the genus *Phytophthora* is responsible for the sudden death of the Live Oak, Tanoak, and Black Oak trees in Coastal California. Fungal infection and beetle tunnels of ambrosia beetles, *Monarthrum scutellare* and *M. dentiger* have been associated with the death of the trees. I studied whether beetles may serve as vectors of the invasive *Phytophthora* fungus. Beetles were tested in partially artificial conditions. Twelve infected logs were cut from Live Oak, Tanoak, and Black Oak trees in Marin water district and incubated in an 80 degree isolation chamber. Beetles that emerged were collected and plated onto *Phytophthora* selected media. Fungal growth was observed and compared to known growths of *Phytophthora*. Approximately 4,000 beetles were tested carrying approximately fifteen different fungi growing on selected media. No definite matches to the known species of pathogen were found. Overall, results of this study did not support the hypothesis that ambrosia beetles vector the invading fungus.

Introduction

For the past five years, Oak trees along the northern California coast line have been under attack of an invasive, non-native, fungus-like oomycete of the genus *Phytophthora*. This fungus and three species of bark and ambrosia beetles have been associated with the oak death, though their roles are still not defined (McPherson et al, 2000). This study focuses on Oak-beetle-*Phytophthora* interactions in the Marin County Municipal Water District. The trees are distributed along 1,500 coast miles in California and Oregon. The spread of the fungal infection is confirmed as far north as Kings Ridge in Sonoma County and as far south as Pfeiffer Big Sur State Park (http://camfer.cnr.berkeley.edu/oaks/.).

Phytophthora ("plant killer") is a genus of plant pathogens with swimming zoospores and thick walled resting clamydospores (Hansen et al, 2000). This pathogenic species was identified in June of 2000 as a *Phytophthora* genus, which is responsible for the Irish potato famine of 1779-1841. In more recent years it has been found to cause of the rapid death of evergreen Oaks in the Mediterranean region and deciduous oaks in Central and Western Europe (Raven et al, 1999). On the west coast of North America the *Phytophthora lateralis* has infected and destroyed thousands of acres of Port-Orford-Cedar in Oregon and Northern California (Hansen et al, 2000). *P. lateralis* is a suspect parent of this new *Phytophthora* strain, potentially caused by hybridization with another *Phytophthora* strain (McPherson, per comm.). Several *Phytophthora* species have been observed to hybridize in alder trees, expressing its habitat flexibility (Brasier, 1999).

The differences between behavior of *P. lateralis* and this new species is that the latter exists only as a root rot and is vectored via soil and water splash, not by wind. It has been shown to survive for seven years in potted soil, but dies within days of being exposed to the sun on the soil surface (Hansen, 1996). The *Phytophthora* infesting the California coast Oaks does not attack subterranean roots but instead infests the soft wood under the bark near the base of the trunk (McPherson et al, 2000). It is not known how the spores are vectored, via soil, water splash, wind, or by beetles. The spawning of the fungus into the sapwood of the trees, bypassing the protective bark layers with remarkable success suggests the fungus is carried into the sapwood by a specialized mobilizing agent which also proliferates in the lower portion of the trunk.

In the case of Sudden Oak Death (SOD) three stages of progressive symptoms have been observed. The first stage is the infection by the Phytophthora in the lower trunk, forming a growth in the cambium layer where it spreads along the surface of the soft wood creating a canker of dark, decomposing wood. This area is identified from the outside by the seeping of the tree, which is caused by the trees defense mechanisms attempting to push out the invading organism. In the second stage, the ambrosia beetle, *Monarthrum scutellare,* the minor Oak ambrosia beetle, *Monarthrum Dentiger*, and the Western Oak bark beetle, *Pseudopityphthorus pubipennis*, colonize the lower trunk in large numbers. They produce reddish-brown piles of dust called frass resulting from their tunneling activity (Palkovsky and Svihra). These beetles are natural parasites to this habitat, usually attacking dead or dying trees. In the case of SOD they are noted to attack green leafed, seemingly healthy but infected trees. The third stage is the growth of hypoxylon fungus growing from the sites of infection. Hypoxylon is a wood decay fungus that commonly feeds from fallen logs or dead standing trees, however it is now found on infected, green to brown leafed trees.

Oak trees are necessary for the existence of ambrosia and bark beetles. The ambrosia beetles tunnel 1-2 ½" into the soft wood, where they lay their eggs and feed from non-aggressive, nutritious fungi. The bark beetles tunnel through the bark, into the cambium where they lay their eggs and feed from the phloem. Here the larvae develop over a period of months. In coastal California, the adult beetles emerge in March-April, with a second brood produced in August-September. The beetles emerge as adults from one host and attack a new, suitable host carrying fungi and microorganisms picked up in the tunnels. When they find a host, they tunnel into the bark and wood, lay eggs inside the cavity, and the cycle continues.

The bark and ambrosia beetles are known to be vectors of several pathogenic fungi. Bark beetles of the *Ips*. spp. have been shown to vector the pitch canker pathogen, *Fusarium subglutinans,* to Monterey Pine in California (Storer et al, unpub). This disease causes mortality, stem deformation, reduced growth, and seedling mortality (Storer et al, 1995). The southern pine beetle, *Dendroctonus frontalis*, infects pine trees with the blue stain fungus, *Ceratocystis minor*, into the phloem where it spreads to the soft and hard wood causing wilting of the tree (Barras, 1970).

There is no information in the literature about the behavior of the beetles after emergence and before host colonization. It is possible that the beetles may land on several trees and even

attempt to bore into a non-optimal tree before finding its final host. The focus of this research was to test whether ambrosia and bark beetles have the potential to vector the *Phytophthora* from infected tree to uninfected tree before finding a suitable host. Demonstrating a new relationship of the beetles with the *Phytophthora* species carries ecological and conservational significance. An understanding into the disease mechanism and aid towards conservation of the oaks can be made. In this experiment beetles were reared from infected logs and independently plated on *Phytophthora* selective media. Cultures suspected to be the new *Phytophthora* were compared to isolates collected from infected trees, using taxonomic and genetic criteria. Fungal cultures that were clearly not Phytophthora were sub-cultured for identification.

Methods

Study Site Infested Oak logs were felled and transported from the Marin Municipal Water District in Northern California and stored in the College of Marin Greenhouse located south of Fairfax. The logs were cut from a flat, moderately forested site, within 20' of Sky Oaks Road and 10' of a stream in an area that contains high levels of precipitation, averaging 35.97 inches per year (http://endeavor.des.ucdavis.edu/newcara).

Sampling Methods Infested trees were cut into 1 ½' - 3' logs and transported to the greenhouse breeding chambers. Two characteristics were required for this experiment: infection by Phytophthora, and invasion by beetles. In the greenhouse, 12 logs were placed in a breeding chamber at 80°F in order to accelerate the process of larval development. The chambers were made out of plywood with sealed doors approximately 4' x 3' x 4'. One of the walls has a Lucite strip running down the center, allowing for light to filter in. This attracted the beetles toward a in the bottom of the bottom of the chamber. A fan at the top circulates the chamber, forcing the beetles toward this hole in the bottom of the unit, which has plastic tubing screwed in to it. The beetles are pushed by gravity feed, through the tubing, down into a containing device, stored in a refrigerator below the chambers.

When the beetles emerged, they were plated every 2-4 days on PARP media and stored at room temperature to allow for fungal growth. The media used to grow the fungus was a *Phytophthora* selected media (PARP). To make one liter of agar, 17g of Difco corn meal agar, and 950ml of distilled water was mixed. This was autoclaved, then put aside to cool when the following antibiotics were added: .4ml Pimaricin (2.5% suspension), .25g Ampicillin

(10ml sterile water suspension), .01g Rifampin dissolved in 1ml DMSO. Suspected fungus growing from the beetles were isolated on to clean PARP plates and observed for *Phytophthora* growth. If the fungus had qualities like those found in fig 1., then they were isolated.

Fig 1. Phytorpthora growing on PARP media
(Photograph courtesy of David Rizzo)

General Identification Eleven isolates of various, non-*Phytophthora* growth have been identified by UC Davis pathologist David Rizzo. He determined Order by typing reproductive structures. Detailed identification was not completed.

Phytophthora Identification UC Berkeley's pathologist, Matteo Garbelotto, used standard PCR technique, which consisted of denaturation of DNA proteins, annealing of known primers (Garbelotto, unpublished), and extension of new DNA (Delidow, et al, 1996). Five isolates of unknown, suspected Phytophthora were tested. Known primers were used to try to amplify complimentary strands of DNA to establish the presence of Phytophthora (Garbelotto, unpublished). If no DNA is amplified than the DNA was not from Phytophthora.

Results

The ambrosia beetles, *M. dentiger* and *M. scutellare*, were the only species to emerge and be plated for this experiment. Bark beetles, *P. pubipennis*, were not present during this experiment. Of the 3,892 ambrosia beetles plated, 15-20 different fungal specimens were isolated from agar and beetles. Of the eleven identified by David Rizzo, Penicillium and various ascomycetes and zygomycetes were observed. Of the five tested using PCR, no positive fungal matches to this

species of *Phytophthora* were made. Exact numbers of different isolated fungal growths is uncertain until further tests into genus and species are completed.

Discussion

The tests showed no significant relationship of the beetles as vectors of the fungus. However, there are considerations that need to be looked at in closer detail. One factor that was not anticipated was the lack of *P. pubipennis* presence. This beetle resides in the cambium layers of the tree, where the *Phytophthora* infection is most successful. It is of particular importance to test this beetle against *Phytophthora* growth because of its spatial association. In addition, it is possible that the bark beetles emerge later than the ambrosia beetles, and therefore were absent during the time frame of the experiment. Another uncontrolled factor was the semi-artificial conditions of the test, as the dehydration of the logs may have caused high mortality of the growing larvae. The moisture content of the phloem would mostly impact the bark beetles because they depend on its nutrients for food, whereas the ambrosia beetles feed on fungal hyphae inside the softwood. Another consideration is that the bark beetles may not be attracted to the *Phytophthora* cankers. Their absence in the logs may be due to a high surface area of infection. A further study into beetle attraction to *Phytophthora* is needed to answer this.

The difference in conditions of spring versus fall environments also plays a role in optimal spore production. *Phytophthora* spore production is most active during stressful periods, which correlate to those found at the end of summer, early fall. An experiment on the beetles during their October flight may contain different results.

There are three experimental considerations that could be changed for optimal results in future studies. One factor is the lag time between beetle emergence and the moment of plating. In this experiment, beetles were plated every 2-4 days. However the average time for a beetle to be flying after emergence and before landing on a potential host averages within a few minutes (Jactel, 1991). The test's strength overall was decreased by the amount of time the fungus had to dry out, be eaten (by either beetles or mites), or fall off the surface of the beetles. For the most accurate results, immediate plating of beetles after emergence, either in the chambers or in nature, is desirable.

The second factor concerns the Phytophthora selective media. Most of the antibiotics are highly unstable compounds and breakdown over a short period of time under room temperature, partial light conditions. Within two months of being plated the media showed unresisting growth

of many fungi and bacteria. Recommendations for future research would be the consistent plating of new media every two weeks, with a one-week lag before its use to allow for partial breakdown of the media, to testable conditions.

The third factor is the method in plating beetles into the media. One hundred percent of the surface area of the beetle should be in contact with the specialized agar. Spore germination is most probable when surrounded by the nutrients of the media. In this study, three quarters of beetles plated were plated this way.

Future Research There may be alternate associations of the beetles and fungus with the Sudden Oak Death syndrome. The physiological impacts of the beetle tunnels in wood have the potential to increase the rate of infection by allowing fungal growth into deep xylem tissue that otherwise is not available. Wood-boring beetles, *Monochamus scutellatus* and *M. motatus*, speed up the rate of decay and death of the heartwood of pine logs by the wood-rotting fungus *Peniophora gigantean* (Leach, et al, 1937) because of the access allowed to the fungus after tunnels have been established. Without the beetles the fungus can initiate invasion of the sap wood, but the heartwood takes much longer. The insect tunnels invade the wood in a linear attack toward the heartwood that allows the fungus to encroach as well, leading to the rapid decay of the whole tree.

Impact of mechanical damage alone can cause the death of the host tree. An example of this was seen during the drought in the late 1980's. The Torrey Pine tree is host to the native five-spined engraver beetle, *Ips paraconfucious*. Under normal conditions the trees' defense mechanism, sap, successfully suppresses beetle outbreak. But the stress of the drought lowered its defenses enough to allow beetle damage to the point of mortality. Approximately 650 trees died in that time. In the case of SOD, attack by ambrosia beetles together with the fungal infection may jointly cause the death of the Oak (www.torreypine.org/tpinsect.htm#BarkBeetle).

It is possible that the ambrosia beetles may not be the vectoring agent of *Phytophthora* spores. This may be because the beetles do not have the mechanisms to do so. A study that will test their ability to carry *Phytophthora* spores could conclude this dilemma.

There are other possible agents that *Phytophthora* can use to move from tree to tree. Important approaches to a study are the impacts of topography, geology, roads, trails, and waterways. More information on the environmental and chemical nature of the *Phytophthora* species would be necessary to learn how best to fight and potentially maintain the infection.

Wind and water are very significant agents of dispersal. However, many fungal pathogen spores are produced in sticky exudates, becoming hard when exposed to the air and drying out (Leach, 1940). Wind is a good agent because it can disperse over a wide range of land, but for these reasons it may not be a probable cause. Rodents and other animals cannot be ignored as potential carriers of the fungus, vectoring by hair or by defecation on the tree. Chlamydospores of *Phytophthora cinnamomi* have been found to survive in the gastrointestinal tract of termites and wild birds (Keast, 1979). Also, a look into this pathogens ability to travel in soil is necessary to know its potential for mobility.

The mechanism behind the association of these two agents still remains a question. Are both agents independently linked by the death of the Oak? For the sake of conservation this comprehension can encourage future researchers to inspect other forms of potential vectors, which would then stimulate methods of preventing the spread of this disease to large areas. Several potential vectoring agents should be studied in order to make significant steps toward managing the spread of this pathogen.

For the purpose of conservation, the difficulty and complexity of studying these organisms proves that preventative measures need to be addressed at the policy level. History has shown that years of research can only pacify a certain amount of ecological chaos. Once the genes are accepted by a habitat there is no chance for removal. Decades of research on the Port-Orford Cedar demonstrate the futility of conservation in the aftermath. The dangers of invasive diseases are real and should cause ecologists in politics to move for increased international restrictions.

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