Interactions between Native Bark and Ambrosia Beetles with *Phtophthora ramorum*

Laura Cattani

Abstract Increasing oak mortality in California and Oregon has been blamed on the pathogen *Phytophtora ramorum*, a disease complex referred to as Sudden Oak Death (SOD). Observations in the field suggest that these oak trees are experiencing higher insect infestation. I researched the association between the oaks and the native bark and ambrosia beetles. I hypothesized that the relationship is enhanced by the presence of *P. ramorum*. While past research has shown no evidence that beetles are spreading the disease, beetle presence in infected trees could weaken live trees to the point of falling over. My research was conducted using a tree from China Camp State Park in Marin County. This infected tree was felled and the trunk was cut into four logs, each about 30cm long. Back in Berkeley, two disks or cookies about 3cm thick were sawed from each log. Using a scalpel, samples were taken from the beetle tunnels, zone lines of the various fungi in the wood, including the *P. ramorum* necrotic areas. 1cm² cells were drawn out around the circumference of each cookie. Beetle holes were counted in each cell and noted as either necrotic or sound wood. Using a Mann-Whitney U-test I compared the mean densities between the healthy and necrotic sections. Results revealed that the necrotic wood exhibited higher density cells than the sound wood. While *P. ramorum* was not isolated from the logs, many other fungi were cultured from the beetle tunnels and zone lines. This suggests that the beetles hasten the invasion of secondary fungi which could significantly weaken the oak trees. Only one tree was available for this study due to busy schedules of the park rangers. As a result, these conclusions do not represent the oak-beetle interaction at large, but do give a solid foundation and reason to do further research.

Introduction

From southern Oregon to Monterey County, increasing oak mortality has been blamed on the pathogen *Phytophtora ramorum* (Garbelotto et al. 2001). Many aspects of this fungus- like oomycete remain a mystery. The disease complex is commonly referred to as Sudden Oak Death (SOD) and affects many oak species as well as other shrubs and trees that inhabit the same ecosystems. Oaks infected with this pathogen experience bleeding cankers on the bark. These cankers are black or brown and seep dark red sap. They occur on the trunk of the tree, up to twenty meters high, but do not infect the roots. Some other symptoms of the disease include black fungus fruiting bodies on the bark, and extreme beetle colonization. The combination of all of these symptoms can cause a tree to die within two years (Garbaletto et al. 2001). The mechanism of spread remains unknown, however many means of dispersal have been hypothesized and tested. A few of these proposed mechanisms are water, people, forest animals, and also insects such as bark beetles (Garbelotto et al. 2001).

Since 1995, SOD has been found in the coastal ranges of California including Alameda, Marin, Mendocino, Solano, Santa Cruz, Monterey, Napa, San Mateo, Santa Clara, Sonoma, and parts of Oregon (McPherson et al., 2002). Because of the devastating nature of this disease, often killing the majority of the trees in an area, these counties are experiencing far- reaching consequences. Oak woodlands are dangerous because of falling trees and limbs and property values are decreasing because of the dead trees (Zentmeyer, 1983). Furthermore, fire danger, wildlife habitat, and aesthetics are becoming important environmental concerns because of SOD (Kan-Rice, 2001). The build up of dead and down wood on the forest floor creates an extreme fire hazard that could burn intense enough as to cause 100% tree mortality in some areas and threaten many homes and communities near these oak woodlands. The crisis is that many trees are falling before the disease has killed them. This could be due to the increased insect infestation in the wood. If the beetles are in fact attracted to *P. ramorum* and weakening the integrity of the wood, then stopping the beetles could help the trees to fight the fungus. As a result of the rapid spread of the disease and its effects on a wide variety of habitats, research is imperative to understand the ecology of this disease. More specifically it is important to understand why these oaks are falling over before they have died.

This study will focus on understanding whether *P. ramorum* attracts beetles to infected wood. Because Marin County is the region hit hardest by SOD, my research will be conducted

using trees from China Camp State Park located on the eastern side of the peninsula. In the park, many stands of oak are experiencing high mortality. Just walking through the woodlands is difficult because of all of the fallen trees. Dave Wood and Brice McPherson have been observing and keeping tabs on about 30 trees throughout the park. They have observed increased beetle frass around bleeding areas on the trunks (personal communication).These observations give reason to believe that beetles are somehow attracted to the necrotic infected wood.

The genus *Phytophtora* is made up of many plant pathogenic water molds that have caused extreme social and economic problems in the past and the present to agricultural crops and ornamental plants (Zentmeyer, 1983). *Phytophtora* seems to do so well because of their flexibility to adapt to the environment. Dispersal of the spores ranges from wind to water to even ants and snails (Carlile, 1983). However, while two species of ambrosia beetles (*Monarthrum scutellare* and *M. dentinger*) and Western Oak bark beetle (*Pseudopityophthorus pubipennis*) have been researched as to being vectors of the pathogen, there is no evidence that shows that the beetles are spreading the disease. Rich (2001) and Mershman (2002) examined the tunneling and gallery establishment of the beetles in infected trees. They were trying to isolate the pathogen from the larvae and/or the gallery walls to show that the beetles could be possible vectors. Their research did not indicate that these beetles were the vectors of the pathogen; however there remains a possibility that an association exists because of many problems that Rich and Mershman ran into in their research. Little is known about the biological requirements of *P. ramorum* and how long it is viable once taken off the host. Destructive sampling methods could have had negative impacts on the survival of the oomycete. Although trees exhibited all of the symptoms of sudden oak death, Rich and Mershman were not able to isolate *P.ramorum* from the cankerous wood. If the wood no longer had viable *P. ramorum* spores, then it is no surprise that the beetles carried no spores.

Other research that has been done shows that there are stages the tree goes through after infestation (McPherson, 2002). First, the tree exhibits bleeding as a defense mechanism against the pathogen. After this, beetles rapidly colonize the healthy wood of the tree around the cankers. When the tree is obviously dying or dead, *Hypoxylon thouarsianum*, a fungal endophyte can be seen on the bark as black fruiting bodies (Garbelotto et al. 2001). Often, trees will fall before the *Hypoxylon* fungus becomes visible and before they are actually dead. This has been observed in many plots at China Camp State Park (McPherson, personal communication).

While there has been increasing research on SOD, there is a lack of research on insect association. Dr. Dave Wood and his lab are currently researching the association between bark beetles and oak trees and whether this association could be enhanced by the presence of *P. ramorum*, and also if the beetles could be spreading the disease to healthy trees. Not only could the beetles be spreading the disease, but also their increased colonization of infected trees could be causing further damage to these oaks. It has been noted in the field that the wood where the trees snap and fall seems to be the area in the tree with the most insect galleries and tunnels (McPherson, personal communication). These areas on infected trees also seem to be healthy wood aside from the beetle colonization. The peculiar thing about the beetle behavior is that they even tunnel into living standing trees exhibiting signs of sudden oak death. These beetles naturally colonize dead wood and usually trees that have fallen (Furniss &Carolin, 1977). The possibility that these beetles are attracted to the infected trees would be logical. There is evidence of volatiles from *Ceratocystis fagacearum,* the pathogen causing oak wilt disease, are insect kairomones that attract beetles to diseased trees (Metcalf, 1987). Insects may have evolved to sense chemicals from host-plant pathogenic responses in order to find habitable wood in which to tunnel and reproduce.

In the field, one can see extreme beetle tunneling around the bleeding cankers of infected trees by the frass or wood dust that shows on the bark. Usually these beetles will not tunnel into live trees and so this evidence of tunneling is very interesting. Also, diseased trees will often break around areas of heavy tunneling. You can see this by the intense beetle galleries on the exposed wood of the fallen trees. In addition to weakening the structural integrity of the oaks, the beetle tunnels may also provide easier entry for other microorganisms and secondary fungus such as *Hypoxylon thoursarium*. While Dr. Wood has plans to research the chemicals involved with the insect-tree relationship, I will investigate the consistent aggregation of the bark and ambrosia beetles to *P. ramorum* infected wood.

My objective is to determine the response of *P. pubipennis, M. scutellare*, and *M. dentiger* to the bleeding cankers on the trunks of diseased oaks. The null hypothesis is that beetles tunnel into necrotic wood and the healthy wood in an even distribution. However, I hypothesize that beetles are attracted to infected wood and so the beetles attack densities are higher in the *P. ramorum* infected areas of the tree, or the bleeding areas than the healthy wood. Also, in an effort to determine how the beetles are weakening the tree, I verify the various secondary fungi

that are in the beetle tunnels. In this way I can ascertain how the beetle tunnels weaken the oak wood.

Methods

The study site was China Camp State Park, which is located in Marin County, California north of San Francisco. The park is on the eastern side of the peninsula along the shores of the San Francisco Bay. The area has a coastal-temperate climate and experiences about 20 in of rain a year (Climate of San Francisco, 2002). The sample will come from areas close to paths and roads for convenience. The rest of the research will be conducted at the Oxford Tract greenhouse in Berkeley on the corner of Oxford and Hearst Avenues.

In order to obtain the logs necessary for the experiment, it was crucial to choose the right type of oak tree. This study focused on coast live oak (*Quercus agrifolia*). The living oak tree was selected with a diameter approximately twenty centimeters with obvious tunneling by *Pseudopityophthorus pubipennis*, *Monarthrum scutellare*, and *Monarthrum dentinger*. Only one oaks tree was available for this study but it was chosen to represent the common attributes of the majority of oak trees in the park. It was determined to be infected with *P. ramorum* by the bleeding cankers on the bark. Also beetle infestation was determined by the obvious fresh bark dust on the trunk, called frass. This frass is created when beetles tunnel in and push out the chewed up bark from their holes. Once the tree was chosen and felled, the trunk was divided into four logs, each about 30 cm long. The tree was cut into sections to make the job more manageable.

These logs were taken across the bay to Dr. Wood's lab in the Oxford Research Unit in Berkeley, California. Here they were put in plastic airtight bags to maintain their moisture. About a week later the logs were removed and a disk about 5 cm thick was cut from each log using a chainsaw. In order to get an unbiased sample from the tree each cookie was taken from the top of each log. On the surface of this fresh wood, there was not only evidence of *P. ramorum* damage to the cambium, but also other fungus that was deteriorating the wood. With a scalpel, material was removed from the bark, inside of the beetle tunnels on the fresh surface, and along the zone lines of the various fungi on the wood, and the wood just inside of the bleeding cankers. The zone lines are the outer edges of the growing fungi that move through the wood. It is best to sample these areas because the fungus here is growing the fastest.

This material was cultured on potato dextrose agar (PDA). Potato dextrose agar is unselective and so most fungi are allowed to grow and multiply, but it is impossible to isolate any one fungus. Because *P.ramorum* was being tested for, a medium called PARP was used after the PDA. PARP is an agar that involves four types of antibiotics that selectively grow *P. ramorum* and kill other fungi and bacteria. Also, to test for other specific fungus an agar with a strong fungicide was used. This fungicide is selective to the Basidioimycete family. After about two weeks, the samples were analyzed. With the help of Bob Raabe I was able to identify the various fungi that were cultured from the disks. The presence or absence of *P. ramorum* was recorded along with other fungus that was present in the beetle tunnels.

After taking samples from the wood, one more set of disks was cut from the logs. This time the disks came from the middle of each log, or 15cm down from the last cut. There were eight disks total for the whole counting portion of the study. Each disk was separated by at least 15cm and contains either one or two necrotic areas. In this was each section on the disk could be considered a different sample. See figure 1.

Figure 1. Sample log. One cookie was taken from the top and fungi samples were taken from the inside. The second cookie was taken from the middle of the log.

First, the disks were debarked with a chisel and rubber mallet. First however, I marked out on each one where the necrotic areas were. As I took the bark off of these areas I marked with my pencil on the wood where the cankers were, which was less obvious without the bark. The necrotic area was irregular and therefore would have been impossible to calculate the surface area to find out the density later on. So, using clear plastic tape that was marked with continuous

1cm² boxes, I wrapped it around each disk, taped it in place, and counted the holes in each box. This way, I was able to categorize each box as either necrotic or sound wood and determine the mean density of holes per square centimeter for each separate necrotic area. See figure 2. After categorizing all of the cells and counting the holes in each one on all eight disks, I was able to compile my data.

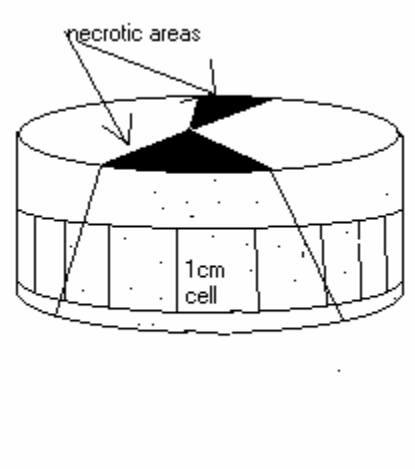

Figure 2. Sample cookie with necrotic areas and 1cm cells marked out.

On each disk there were different sections of the wood that were either necrotic or healthy. This means there were either one or two cankerous areas on each disk. Each section was considered as a separate sample. First I counted the 1cm² cells that made up each section. Then I counted total holes for each section inside the cells. In this way, I divided total holes by number of cells and arrived at a density of holes/ cm² for each separate necrotic and healthy area. Then I was able to compare the mean densities between all of the necrotic areas and all of the healthy areas. I used the Mann-Whitney U-test because the data was not normal and it was necessary to use a non parametric test.

Results

The following table shows the number of cells, number of holes and density for each necrotic and healthy area on each cookie

Cookie	Section	Cells	# of holes	Holes/cm²
1-A	Necrotic	28	28	1.00
1-B	Necrotic	8	11	1.38

	Healthy	3	2	0.67
	Necrotic	11	12	1.09
	Healthy	8	1	0.13
	Necrotic	3	6	2.00
1-C	Healthy	3	0	0.00
	Necrotic	9	13	1.44
	Healthy	23	2	0.08
	Necrotic	4	0	0.00
1-D	Healthy	35	4	0.11
2-A	Necrotic	37	46	1.24
2-B	Healthy	4	6	1.50
	Necrotic	3	5	1.67
	Healthy	6	4	0.67
	Necrotic	25	34	1.36
2-C	Healthy	7	1	0.14
	Necrotic	22	22	1.00
	Healthy	7	0	0.00
2-D	Healthy	15	2	0.13
	Necrotic	5	3	0.60
	Healthy	21	2	0.09

Table 1. Densities of beetle holes for each section on each cookie.

There were significantly more beetle holes in the necrotic sections of the tree (1.16 ± 0.53 sd) than in the healthy area (0.32 ± 0.45 sd; U= 101, n1 = 11, n2 = 11, P= 0.008)

In addition to the beetle tunnels, the cultures taken from the tunnels and zone lines in the wood did not reveal any evidence of *P. ramorum*. However many other fungi were found. These fungi are summarized in Table 2.

Cookie	Culturing medium	Fungi	Where found
1-A	PARP	*Trychoderma*	Zone line
	Fungicide agar	*Penicillium*	Tunnel
1-B	PARP		
	Fungicide agar	*Zygomycete*	Tunnel
1-C	PARP	*Aspergillus*	Zone line
	Fungicide agar		
1-D	PARP	*Ceratocystis*	Zone line
	Fungicide agar		
2-A	PARP	*Trychoderma*	Zone line
	Fungicide agar		
2-B	PARP	*Trychoderma* *Aspergillus* *Penicillium*	Zone line
	Fungicide agar	*Penicillium*	Tunnel
2-C	PARP		

	Fungicide agar	*Zygomycete*	Tunnel
2-D	PARP	*Ceratocystis*	Zoneline
	Fungicide agar		

Table 2. List of fungi from the oak tree and where each was found on the cookie.

Discussion

This oak tree exhibited significantly higher density of beetle holes in the necrotic wood than in the healthy wood as shown by the low p-value. This confirms my hypothesis that beetles are more likely to tunnel into wood that has been infected with *P. ramorum* than healthy wood. With more research this could even mean that beetles are more likely to tunnel into infected trees before healthy trees even if the infected trees are still alive and standing. This is pertinent information to the struggle to manage oak woodlands in California. If the beetles could be controlled with some sort of integrative pest management, then these valuable oak trees could be given a longer chance to fight the *P. ramorum* pathogen. The relationship between *P. ramorum* and ambrosia beetles parallels the similar interaction between ambrosia beetles and beech bark disease in England (Speight, 1981). First, the beech trees are infested by scale insects (*Cryptococcus fagisuga*) and then fungus (*Nectria spp*.), and finally the ambrosia beetles attack the dying trees. The ambrosia beetles (*Xyloterus domesticus*) tunnel in, produce galleries in the wood and weaken the trees to the point of causing the tree to snap and fall.

While this information reveals a lot about the ecology of *P. ramorum*, it can only be applied to this individual tree. Only one tree was sampled and so it is impossible to use this as a representation of oaks in China camp, much less all over SOD territory. However, it does bring to light very interesting observations for future research. In this study I was able to quantify the interesting observations made in the field (Wood, McPherson, personal communication). While it is impossible to statistically determine, the tree that we brought in from the field is very similar qualitatively to many other trees in China Camp State Park in that it displayed frass around the necrotic, bleeding cankers on the trunk. Possibilities to improve this study include a larger sample of trees from all over the park. With a larger number of trees, the data could be stronger and more robust in determining the positive relationship between *P. ramorum* and ambrosia beetles, *M. scuttelare* and *M. dentiger.*

While Mershman (2002) and Rich (2001) showed that these beetles are probably not involved in pathogen dispersal, they are most definitely opportunistic organisms, which hasten the death

of the host. The material taken from the necrotic wood did not yield *P. ramorum*. This is most likely due to the fact that *P. ramorum* becomes inviable after only a few days. However, much of the biological requirements are still unknown for the oomyecete. Also, the various other fungi in the wood are better adapted to oak wood and so give *P. ramorum* hard competition for resources such as nutrients and oxygen. As stated earlier, the tree I studied had all of the symptoms of sudden oak death but did not yield *P. ramorum*. The bleeding cankers on the trunk signify that the tree was infected with *P. ramorum* and yet wood samples taken directly from the cankerous areas did not yield the oomycete. This shows how difficult it is to culture to oomycete when so much of the biological requirements are unknown.

The interesting zone lines of secondary fungus show that there are many other fungi that have entered the wood either because of the beetles or because the tree had been weakened by *P. ramorum* and made it more susceptible to other pathogens. The evidence of secondary fungus around the beetle tunnels shows that perhaps other pathogens are moving into the tree through the beetle tunnels. Supposing these trees are colonized by beetles at a higher rate because they are infected with *P. ramorum*, this suggests that the beetles are weakening the wood by spreading secondary fungi, like the ones observed by the wood samples. Future research would also include quantifying exactly how much the secondary fungi are weakening the otherwise healthy wood outside of the necrotic area.

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