

# Consequences of *Phytophthora ramorum* Infection in Coast Live Oaks<sup>1</sup>

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## Abstract

Sudden oak death, caused by *Phytophthora ramorum*, has infected and killed large numbers of oaks (*Quercus* spp.) and tanoaks (*Lithocarpus densiflorus*) in California since the mid 1990s. Since March 2000 we have been investigating the interactions between patterns of disease progression and broader landscape-scale patterns of disease incidence and expansion in study plots in Marin County. The incidence of new infections has continued to increase in both coast live oaks (*Q. agrifolia*) and California black oaks (*Q. kelloggii*), and very rapidly in tanoaks. The net result of more than 10 years of *P. ramorum* presence in these forests has led to dramatically altered overstory structure. Larger diameter trees are more likely to be infected and once infected, are more likely to be attacked by beetles. Beetle attacks dramatically lowered the estimated median survival of infected coast live oaks, from 7 years to less than 3 years, with comparable results for tanoaks. In 2001 we established landscape-scale monitoring in Marin and Santa Cruz Cos. to assess spatial and temporal dynamics of disease spread within vegetatively heterogeneous forests. In Marin Co., the proportion of coast live oaks that was infected decreased by 2004, while the proportions increased in tanoaks and Shreve oaks (*Q. parvula* var. *Shrevei*) in Santa Cruz Co. by 2005. To evaluate the role of bark and ambrosia beetles associated with this disease in coast live oaks, we inoculated asymptomatic trees with *P. ramorum*, used an insecticide to experimentally prevent beetle attacks, and placed traps on inoculated and wounded (uninoculated) trees. Traps on infected trees caught 97 percent of all beetles trapped. The size of the bleeding cankers was positively correlated with the response of beetles to infected trees. Both the number of beetles trapped on infected trees prior to any attacks and the intensity of beetle attacks predicted progression to advanced disease stage later in the year. We conclude that beetles naturally adapted to infest recently killed oaks are exploiting a new resource and in the process are accelerating the rate at which *P. ramorum* kills these trees.

**Keywords:** *Phytophthora ramorum*, *Quercus agrifolia*, *Quercus kelloggii*, *Lithocarpus densiflorus*, bark and ambrosia beetles.

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## Introduction

The causal agent of sudden oak death was determined to be a *Phytophthora* species by summer 2000 (Rizzo and others 2002). However, this pathogen, *P. ramorum*, was probably present in coastal California forests since the early 1990s. A large proportion of the woody plant species in these forests are hosts (Rizzo and Garbelotto 2003), but because oaks and tanoaks (*Lithocarpus densiflorus*) are more consistently killed than any other species, the epidemic has probably been a significant factor in changes in forest composition for at least 15 years in the most heavily impacted areas. Any comprehensive evaluation of its impact is constrained by the fact that significant damage had already been done by the time this pathogen was found in these forests. As a consequence of this delay in initiating studies of its effects, there is considerable uncertainty in reconstructing subsequent changes in the species composition of these forests, as well as their ecology. This paper presents a summary of our research on the disease known as sudden oak death and attempts to synthesize some results from these various studies.

In March 2000, we established 20 plots in Marin Co. to better understand the nature of this unidentified disease that was killing large numbers of oaks and tanoaks. Our field observations suggested the involvement of a pathogen in the disease syndrome, but in the absence of a confirmed causal agent, speculation included such factors as acid precipitation, air pollution, aerial application of herbicides, and ill-defined “oak decline”.

The principal limitation of these disease progression plots is that the results cannot be reliably extrapolated to watersheds or landscapes, because these plots were not randomly placed, and thus may not be representative of other sites. In order to obtain estimates of disease behavior that might be more broadly applicable, in June (Marin Co.) and October (Santa Cruz Co.) 2001, we initiated landscape-scale studies to evaluate the extent and distribution of the disease in oaks and tanoaks.

Extensive tunneling by ambrosia and bark beetles restricted to *P. ramorum*-induced cankers is common on infected oaks and tanoaks, but is not consistent with previously reported behaviors of these insects (Chamberlain 1958; Furniss and Carolin 1977). In July 2002, we initiated experiments to evaluate the possible roles of bark and ambrosia beetles, by inoculating coast live oaks (*Q. agrifolia*) with *P. ramorum*, applying insecticide to half the trees, and monitoring beetle responses to trees and tree responses to the different treatments.

The studies summarized here focus on interrelated facets of the disease, primarily in coast live oaks. The goal of the disease progression study was to determine the time course of the disease, the involvement of associated secondary organisms, and any characteristics of the host tree that affected the likelihood of infection and its outcome. The goal of the landscape-scale study was to use an unbiased spatial sampling method to map the distribution of the disease and its impact across two different forest types with patchy species distributions, to monitor change over time, and to determine if any quantifiable environmental variables could be used to predict disease incidence and severity.

## Methods

### ***Disease Progression.***

Twenty disease progression plots were established in Marin Co. in March 2000, ten each in China Camp State Park (CCSP) and in Marin Municipal Water District (MMWD). We monitored every coast live oak, black oak (*Q. kelloggii*), and tanoak larger than five cm dbh (diameter at breast height) in each plot (mean number of trees = 48.3, Standard Error = 2.6) four times per year through 2003, then twice every year thereafter. Symptoms of sudden oak death and associated secondary organisms were recorded, including bleeding, beetle attacks, and reproductive structures of *Hypoxylon thouarsianum*, a fungus that consistently appears in the latter stages of the disease. Data were analyzed for relationships among the primary observable disease symptom of bleeding and the variables beetle attacks and stem dbh. Survival analysis was conducted using a Weibull regression model to identify variables that were associated with increasing probability of tree mortality, for both coast live oaks and tanoaks.

### ***Landscape-scale Studies***

In 2001, we placed an array of linear transects in China Camp State Park, where the dominant hosts are coast live oaks and black oaks, and in Soquel Demonstration State Forest (SDSF), where tanoaks and Shreve oaks (*Q. parvula* var. *shrevei*) are the dominant hosts. The *P. ramorum* epidemic has been characterized by a patchy distribution of infected areas within noninfected forest stands (Rizzo and Garbelotto 2003). We established a series of permanent sampling transects to collect detailed measurements of disease incidence in oaks and tanoaks, as well as the associated vegetation. The point-centered-quarter density estimation method (Engemann and others 1994) was chosen to permit us to cover large areas of land, with minimal expenditure of time spent at each sampling location. On each site, we placed parallel transects 0.5 km apart, with sampling nodes every 100 m. The distance, azimuth, dbh, and disease status were recorded for the closest oak or tanoak in each of four cardinal directions at each sampling node, and reevaluated in 2004 (CCSP) and in SDSF during 2003 and 2005. In addition, at each node, we used a 10-factor prism to provide an estimate of the basal area of all woody stems, by species. Transect length varied considerably within each site, with a total of 95 nodes in CCSP and 135 in SDSF.

### ***Beetle Response Study***

Two coast live oak-dominated sites in Marin Co. were selected in 2002 to study the effects of beetle attacks on disease progression. A total of 80 coast live oaks were inoculated by removing a bark disk, placing an agar plug of *P. ramorum* directly on the sapwood surface, then replacing the bark. Another 40 trees were wounded but not inoculated, to control for beetle attraction to wounds alone. The synthetic pyrethroid insecticide permethrin was applied to half the trees in each group to control beetle attacks. Beetles were collected periodically in 2003 from sticky traps hung on each insecticide-treated tree and were analyzed by collection date, tree treatment status, size of the canker, and disease stage in December 2003.

## Results

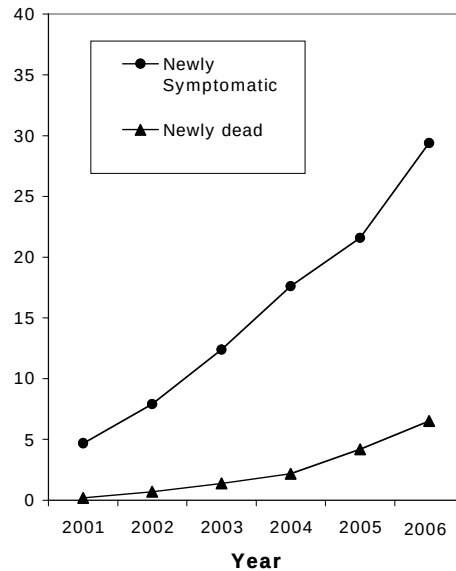
### *Disease progression*

In the disease progression study we found that large proportions of the host trees were symptomatic in 2000, with the incidence of infections approximately stable through 2006 in coast live oaks and increasing in black oaks and tanoaks (*table 1*).

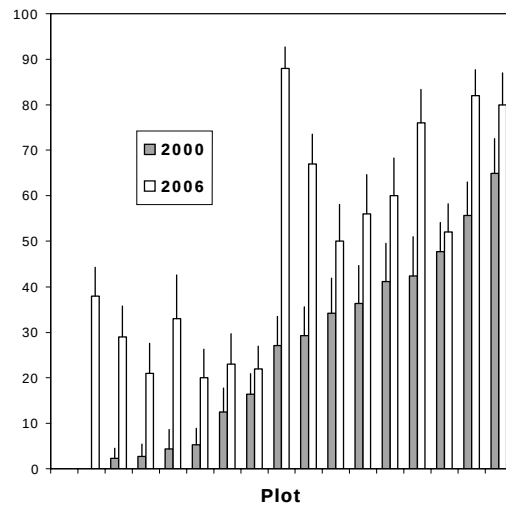
*Table 1—Percentages of oaks and tanoaks in Marin Co. disease progression plots that were bleeding or dead with bleeding, from 2000 through 2006.*

| Host Species   | 2000<br>Percent Bleed/Dead<br>with bleeding | 2003<br>Percent Bleed/Dead<br>with bleeding | 2006<br>Percent Bleed/Dead<br>with bleeding |
|----------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Coast live oak | 25.0 / 5.8                                  | 23.6 / 17.4                                 | 25.0 / 22.9                                 |
| Black oak      | 15.7 / 3.8                                  | 25.0 / 9.4                                  | 36.2 / 13                                   |
| Tanoak         | 39.0 / 8.3                                  | 62.4 / 22.2                                 | 75.9 / 32.0                                 |

The proportion of newly symptomatic coast live oaks has increased at a steady rate in the plots since the initiation of the study (*fig. 1*). A plot of the total disease impact (defined as the percentage of trees that are symptomatic and dead with evidence of bleeding) shows that sudden oak death continues to have severe effects on this species in Marin Co. (*fig. 2*).



**Figure 1**—Cumulative percentages of newly symptomatic and dead coast live oaks, determined for each March sampling date, 2001 through 2006.



**Figure 2**—Combined proportion of coast live oaks that were bleeding and dead, by plot, for China Camp State Park, in 2000 (closed bars) and 2006 (open bars).

Beetle attacks have been consistently detected in 40 to 60 percent of bleeding coast live oaks in every year since 2000. The median Weibull survival estimated for bleeding coast live oaks in CCSP that had not been colonized by beetles was 7.0 (1.2) y, decreasing to 2.6 (0.3) y for trees that had been attacked by beetles, with similar results for tanoaks (McPherson and others 2005). For coast live oaks, black oaks, and tanoaks, dbh was positively correlated with the probability of developing infection. Larger diameter infected coast live oaks were more likely to be attacked by beetles. Extensive beetle tunneling was found in 93 percent of the bleeding coast live oaks, both living and dead, that failed on the main stem. This study documented the consistent association of bark and ambrosia beetles with diseased coast live oaks and black oaks and their near-ubiquity in those that eventually died.

### ***Landscape-scale Studies***

In both landscape-scale studies, (Marin and Santa Cruz Cos.), we found that the percentage of coast live oaks that were symptomatic declined between 2001 and 2004 (Marin Co.) and 2005 (Santa Cruz Co.) (*tables 2 and 3*). In contrast, the percentage of infected tanoaks and Shreve oaks increased in Santa Cruz Co. Tree death, the ultimate measure of the impact of this pathogen, doubled in coast live oaks from 2001 to 2004 (Marin Co.) and increased sharply for all species by 2005 (Santa Cruz Co.). Through 2004, between 50 and 60 percent of bleeding coast live oaks in Marin Co. were attacked by beetles.

**Table 2**—China Camp State Park, point-centered quarter sampling of symptomatic and dead trees, 2001 and 2004. Shown are percentages of living trees (Bleeding) and percentages of all trees (Dead).

| Species                  | Percent Bleeding |      | Percent Dead with Bleeding |      |
|--------------------------|------------------|------|----------------------------|------|
|                          | 2001             | 2004 | 2001                       | 2004 |
| Coast live oak (n = 364) | 22               | 15   | 6                          | 16   |
| Black oak (n = 52)       | 24               | 28   | 15                         | 25   |

**Table 3**—Soquel Demonstration State Forest, point-centered quarter sampling. Categories are the same as in Table 2.)

| Species                 | Percent Bleeding |      |      | Percent Dead (all trees) |      |      |
|-------------------------|------------------|------|------|--------------------------|------|------|
|                         | 2001             | 2003 | 2005 | 2001                     | 2003 | 2005 |
| Tanoak (n = 406)        | 14               | 15   | 29   | 2                        | 2    | 11   |
| Shreve oak (n = 100)    | <2               | 2    | 22   | 1                        | 6    | 17   |
| Coast live oak (n = 26) | 15               | 16   | 8    | 0                        | 4    | 14   |

In China Camp State Park, the mean annual percentage of coast live oaks that became newly symptomatic was somewhat higher in the disease progression plots, 3.7 percent, than in the plotless landscape-scale evaluation, 1.6 percent. A comparison of infection levels in the two forests suggests that the epidemic began later in SDSF and may still be in the expansion phase.

### **Beetle Response Study**

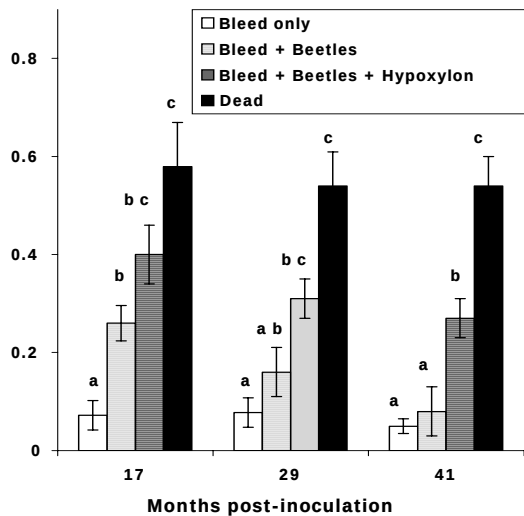
The inoculation of asymptomatic coast live oaks produced the disease symptoms and variation in their expression observed in naturally infected trees. This was reflected in the variation in canker size among trees, from 0.008 m<sup>2</sup> to 1.13 m<sup>2</sup>. Beetles attacked five percent of the inoculated trees within three months of inoculation, prior to the development of bleeding. Although permethrin was applied to half the trees with the expectation that beetle attacks would be prevented, the deterrent effect only lasted about two months after the February 2003 treatment. However, even after beetles breached the insecticide barrier, the mean number of attacks per permethrin-treated tree remained lower than on the untreated trees (Repeated measures ANOVA:  $F_{1,53} = 6.4$ ,  $P = 0.014$ ). Through 2003, the proportion of trees attacked and those that died did not differ between the two treatment groups.

Traps on inoculated trees accounted for 97 percent of all beetles caught in 2003. At each of four sampling dates, beetle counts were greater for inoculated than mock-inoculated trees (Repeated measures ANOVA:  $F_{1,52} = 12.56$ ,  $P = 0.001$ ). Traps on inoculated trees that developed bleeding caught significantly more beetles than those that had never shown bleeding. All the beetle species trapped are classified as saprotrophs and are reported to be restricted to dead trees or dead parts of living oaks. Most of the trapped beetles were in the family Scolytidae, including the ambrosia beetles, *Monarthrum scutellare* (the most abundant species), *M. dentigerum*, *Xyleborus californicus*, *Xyleborinus saxeseni*, and *Gnathotrichus pilosus*, a bark

beetle, *Pseudopityophthorus pubipennis*, and *Scobicia declivis* (family Bostrichidae).

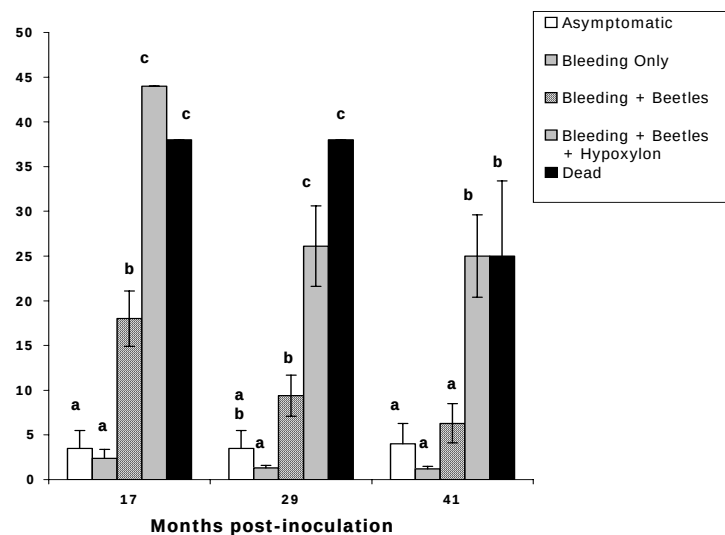
The trap catch in April was significantly greater on trees with larger cankers, prior to the first beetle attacks on the permethrin-treated trees (ANOVA:  $F_{1,23} = 6.85$ ,  $P = 0.015$ ). Once beetles attacked the treated trees, subsequent cumulative trap catches were significantly correlated with numbers of entrance holes (repeated measures ANOVA:  $F_{1,20} = 29.45$ ,  $P < 0.0001$ ) but not with canker size.

Canker size in April 2003 was significantly correlated with advanced disease stage (beetle attacks and fruiting structures of *H. thouarsianum*) by December 2003 and in the next two years (ordinal logistic regression,  $P < 0.0001$ ). The trees that died by December 2005 had the largest mean canker size, followed by living trees with beetles plus *Hypoxylon*, and by those that had been attacked by beetles (*fig. 3*).



**Figure 3**—Canker size measured ten months following inoculation predicted the severity of disease stage in inoculated trees at 17 (2003), 29 (2004), and 41 (2005) months post inoculation. Columns for each date labeled with different letters are different ( $P < 0.05$ ).

The numbers of beetles trapped in April, prior to the first beetle attacks on these trees, showed a similar positive relationship with advanced disease stage (*fig. 4*). The trees that attracted the most beetles were those that subsequently were attacked in the greatest numbers and that progressed to advanced disease, and to death.



**Figure 4**—Mean trap catch in April 2003, ten months post inoculation, predicted disease severity, categorized by advanced disease stage. Columns for each date labeled with different letters are different ( $P < 0.05$ ).

## Discussion

The disease known as sudden oak death is now understood to be less sudden and less consistently deadly (Garbelotto and others 2003; McPherson and others 2005) than was initially believed. The consequences for infected forests are nonetheless considerable, having already led to significant losses of oaks and tanoaks, trees whose overall ecological importance in these forests is probably greater than the other host species of *P. ramorum*. Improved understanding of the dynamics of this epidemic will come from studies at different spatial scales that integrate the effects of the insects and fungi that follow the initial infection. It is clear that *P. ramorum* infections in oaks and tanoaks cannot be understood solely as a pathogen-tree interaction and that sudden oak death may be best described as a disease syndrome involving the pathogen, insects, and fungi.

The rate at which populations of asymptomatic coast live oaks develop infections in the Marin Co. plots does not appear to have declined since 2000. This result was unexpected, as observations of forest canopies for several years after 2001 showed fewer recently killed trees and the percentage of infected living trees has remained relatively stable (table 1). However, the approximately constant rate of new infections (fig. 1) suggests that although large numbers of the least resistant trees may have been infected early in the epidemic, many of the remaining trees are also susceptible to infection.

The inoculated trees exhibited considerable variation in bleeding canker size, a phenomenon that has been interpreted as reflecting resistance to *P. ramorum* (Rizzo and others 2002; Dodd and others 2005). Resistance may be a transient property of the host. If climatic variation or the activities of such biotic factors as insects and



fungi shift the balance toward the pathogen in different years, the disease may progress more rapidly even in trees with smaller cankers. The 3.7 percent average annual rate at which coast live oaks in the Marin Co. study have become newly infected since 2001 forecasts very heavy losses of these trees in years to come. We have shown that *P. ramorum* is more likely to infect larger trees and that, once infected, larger trees are more likely to be attacked by beetles. Since beetle attacks are shown to significantly reduce the median survival of these trees, the production of acorns will be significantly reduced as the epidemic removes larger trees from the forests. The presence of very few seedling or sapling coast live oaks or black oaks in these forests (unpublished observation) indicates that little recruitment was occurring prior to the epidemic. Although seedlings and saplings of the true oaks do not appear to be readily infected (Rizzo and others 2002), there may be few mature specimens remaining after another 25 years with *P. ramorum* as a component of the forest biota. Shifts in species dominance will likely lead to cascading ecological consequences wherever *P. ramorum* has become established in forests with a large component of these or other susceptible oak species.

One factor that emerges from these studies is the consistent role of bark and ambrosia beetles in the weakening and death of infected oaks. Although these beetles are understood to be saprotrophic, restricted to moribund or recently killed oaks, their attacks on *P. ramorum* cankers occur within one to two years following infection, when the foliage appears vigorous and the trees exhibit no visible indications of the general decline expected for trees that are suitable ambrosia beetle hosts. While *P. ramorum* alone causes damaging cankers that may severely compromise tree health by interfering with phloem and xylem transport (Parke and others 2006), beetles that penetrate deep into sapwood may irreversibly disable the defenses of the trees.

These responses of beetles to *P. ramorum*-infected trees may be mediated by volatile compounds released by cankers. The size of the canker may affect the quantity of attractant volatiles released, as well as the surface area suitable for beetle tunneling. Once beetles have tunneled into a tree, their pheromone production likely becomes the dominant attractant for other beetles (Wood 1982; Paine and others 1997). Greater numbers of beetles in a tree will likely lead to increased damage to both phloem and sapwood, as well as the introduction of various decay fungi deep into the sapwood (Svihra and Kelly 2004). Since beetles had attacked approximately half of the coast live oaks in both naturally infected and artificially inoculated stands during any observation period, these insects must be seen as components of the disease syndrome. Once beetles initiate attacks on an infected tree, their pheromone production probably leads to an irreversible decline in the health of the tree as beetle attacks increase.

The landscape-scale and plot studies are demonstrating that sudden oak death continues to change the ecological roles of susceptible oak species and tanoaks in coastal California forests. The beetles that follow the infection are secondary in temporal sequence, but may significantly inhibit the ability of infected trees to defend against the pathogen. Studies presently under way in our lab should better define the sequence of beetle and fungal activity in infected coast live oaks and help to clarify their roles in tree death.

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