Disease Risk Factors and Disease Progress in Coast Live Oak and Tanoak Affected by *Phytophthora ramorum* Canker (Sudden Oak Death)[1]

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Abstract

This paper reports on five years of observations in a case-control study examining the role of tree and site factors on the development of *Phytophthora ramorum* stem canker (sudden oak death) in coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). In September of each year from 2000 through 2004, we collected data on *P. ramorum* symptoms, tree condition, midday stem water potential (SWP), and various other factors in 150 circular plots (8 m radius). Each plot was centered around a case (symptomatic) or control (asymptomatic) plot center tree. Plots were located at 12 locations in the California counties of Marin, Sonoma and Napa in areas where *P. ramorum* canker was prevalent in 2000. Between September 2000 and September 2004, the percentage of symptomatic coast live oak trees in the plots increased slightly, from 23 percent to 24 percent. Over the same period, the percentage of symptomatic tanoaks increased from 31 percent to 43 percent. Between 2000 and 2004, mortality due to *P. ramorum* increased from 4 percent to 9 percent in coast live oak and from 12 percent to 23 percent in tanoak. About 58 percent of coast live oak and 47 percent of tanoak study trees with disease symptoms in 2000 progressed to a more advanced disease severity class by 2004. In both species, some symptomatic trees developed callus tissue along at least part of the canker margin where canker expansion was apparently inhibited. In some infected trees, cankers have not changed in size for several years and appear inactive. Diffuse canopy dieback commonly develops in coast live oaks with advanced *P. ramorum* canker symptoms that survive for at least several years. Most coast live oaks and tanoaks with *P. ramorum* canker symptoms have maintained relatively high stem water potential (SWP) levels and do not show progressive increases in water stress as disease develops. For coast live oak, trees with low water stress (high SWP) are more likely to develop *P. ramorum* canker, but subsequent disease progress in symptomatic trees is not related to SWP. Only two of numerous bark characteristics were associated with *P. ramorum*

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canker in coast live oak. The presence of unweathered, brown bark in bark furrows was the only bark surface characteristic that was positively correlated with disease. This characteristic may be associated with faster rates of bole radial growth, and is consistent with other analyses indicating that faster-growing coast live oaks may have a greater risk of developing *P. ramorum* canker than slow-growing trees. Bark thickness was positively correlated with both the risk of developing *P. ramorum* canker and the likelihood of disease progress among infected trees. Because bark thickness also increases with stem diameter, it is possible that the lack of cankers on small coast live oak stems and branches could be related to their relatively thin bark.

Key words: bark morphology, bark thickness, *Phytophthora ramorum,* resistance, stem water potential, sudden oak death, water stress

Introduction

Phytophthora ramorum, the causal agent of sudden oak death, causes bleeding bark cankers on the main stems of tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and several other oak species in California (Garbelotto and others 2001, Rizzo and others 2002). The bark cankers can expand over time and eventually girdle susceptible trees. The sapwood-decaying fungus *Hypoxylon thouarsianum*, ambrosia beetles (*Monarthrum* spp.), and oak bark beetles (*Pseudopityophthorus* spp.) are commonly associated with *P. ramorum*-infected trees in later stages of decline (Garbelotto and others 2001).

We initiated a long-term study to follow disease progress and evaluate disease risk factors in the summer of 2000, shortly after *P. ramorum* (then unnamed) was identified as the cause of sudden oak death. Most of the study trees are coast live oak, but we collected parallel data on tanoak at two locations for comparative purposes. We used a case-control study design to test whether various tree factors and plot/stand factors were related to the development of *Phytophthora* bole cankers in coast live oak in areas where the disease was common. Models based on results from the first three years of this project (Swiecki and Bernhardt 2001, 2002a,b) were the first to document that California bay laurel (*Umbellularia californica*) cover and density near coast live oak is significantly correlated with disease risk. Other predictors of disease risk in coast live oak included canopy dominance (canopy sky exposure), plot canopy cover, stem water potential (SWP, a measure of water stress), tree decline associated with other agents, stem diameter, and multiple main stems. Based on the effects of predictors related to tree growth rate, we inferred from these models that trees with faster growth rates (associated with larger diameter, higher

SWP, greater sky exposure, lack of decline from other agents) had an elevated risk of *P. ramorum* canker.

By collecting data on disease status over time in these plots, we have been able to determine how the disease status of these trees has changed over time, improving our overall concepts of disease risk and disease progress in this population. In addition, between 2000 and 2004 we have collected data on additional variables that were not considered in the original study design. In particular, bark morphology characteristics, including bark thickness, have been included in analyses reported in this paper.

Repeated observations on the trees in these study plots over the past five years has also provided detailed information on long-term symptom development and disease progress in this cohort. These observations include ratings of canker expansion, the abundance of *H. thouarsianum* stromata and beetle boring, changes in canopy health, branch and main stem failures and September water stress levels, as indicated by SWP. From these observations, we have been able to document patterns of disease progress that were not apparent when sudden oak death was originally described in California (Garbelotto and others 2001, Rizzo and others 2002).

Methods

Plot selection

The methods used to select study locations and plots have been previously described (Swiecki and Bernhardt 2002a). During September 2000, we established plots and collected data at 12 study locations (*table 1*). Locations were chosen in areas where *P. ramorum* was prevalent at that time.

At each study location, we collected data in circular 8 m radius (0.02 ha) plots, each of which was centered around a case or control tree. Case trees had only early symptoms of *P. ramorum* canker (bleeding cankers), with the exception of nine coast live oak cases that had minor amounts of beetle boring or a few small *H. thouarsianum* stromata, typically on a single scaffold or on a localized portion of the bole. Control trees were lacking any *P. ramorum* canker symptoms. In 2000, we established 128 coast live oak plots (53 cases, 75 controls) and 22 tanoak plots (10 cases, 12 controls).

Table 1— *Locations of plots and host species studied*

Location number	Location	County	Number of plots	Tree species
1	Marin Municipal Water District (MMWD) watershed - Azalea Hill area	Marin	12	coast live oak
2	MMWD-Pumpkin Ridge south	Marin	16	coast live oak
3	MMWD-Pumpkin Ridge north	Marin	11	coast live oak
4	MMWD-Phoenix Lake area	Marin	11	coast live oak
5	China Camp SP - Miwok Meadows area	Marin	16	coast live oak
6	China Camp SP - SE Buckeye Point area	Marin	12	coast live oak
7	Woodacre (Private land)	Marin	12	coast live oak
8	Lucas Valley (Private land)	Marin	12	coast live oak
9	Muir Woods NM / Mt. Tamalpias SP	Marin	10	tanoak
10	Wall Road (Private land)	Napa	13	coast live oak
11	Marin County Open Space land, Novato	Marin	13	coast live oak
12	Jack London SP	Sonoma	12	tanoak

Disease ratings

Trees in plots were evaluated each year in September of 2000 through 2004. Tree species were rated as trees if they had at least one stem 3 cm in diameter at 137 cm height (DBH).

For all canker host trees (coast live oak, tanoak, and California black oak) in the plots, we noted whether *P. ramorum* canker symptoms were present on the main stems and the stage of disease development. We did not assess tanoaks for the presence of *P. ramorum* twig cankers. Trees were classified as having early symptoms if only bleeding cankers were present. We defined late disease symptoms as including cankers and *H. thouarsianum* stromata and/or beetle boring. Trees killed as a result of *P. ramorum* cankers were classified as the dead *P. ramorum* symptom class. Initially symptomatic trees that showed no bleeding or other evidence of disease progress for at least two consecutive years were classified as having inactive infections if cankers were still visible and were rated as asymptomatic if external canker symptoms had disappeared. Severe decline (poor condition likely to result in tree death within 10 years) and death due to agents other than *P. ramorum* (primarily canker rot fungi) were also evaluated for each tree.

Disease ratings were based primarily on visual assessments. Chipping of bark to expose or sample cankers was minimized to avoid affecting future observations on the study trees. For some symptomatic trees, tissue pieces were placed into PARP semi-selective media (Erwin and Ribeiro 1996) to isolate the pathogen. Identifications of cultures were made by specialists at the lab of David Rizzo (University of California, Davis). Isolations were primarily restricted to atypical cankers and new cankers that appeared after 2000. In a few instances, isolations attempted in successive years have yielded negative results one year and positive results the next year. Because isolations can result in false negative results, especially in trees with older cankers sampled late in the season, trees with very typical *P. ramorum* cankers were rated as symptomatic even if isolation results were negative. For a few trees with ambiguous disease symptoms, tree disease symptoms in later years of the study were used to retrospectively reassign disease status for data analysis. The majority of these reassignments were trees rated as symptomatic in 2000 that were reclassified as asymptomatic due to a lack of symptoms in all subsequent years.

We collected additional detailed information on the extent of *P. ramorum* canker symptoms in plot center trees and plot trees used for additional water potential measurements (extra SWP trees, see below). Trunk girdling by *P. ramorum* cankers, *H. thouarsianum,* and beetles were scored by estimating the percent bole circumference girdled (0 to 6 scale described below) as if all affected areas were projected onto the same cross section. In 2003, we also recorded the apparent height above the soil of the lower margin of the lowest *P. ramorum* canker and the upper margin of the highest *P. ramorum* canker for coast live oak trees only. Bleeding, externally visible bark symptoms, and in some trees, limited chipping of outer bark, were used to delimit the extent of cankers. We also used the number of bleeding areas to estimate the number of *P. ramorum* cankers present each year.

We also assessed overall (chronic) canopy thinning (none, slight, definite), the presence of epicormic branches (none, few, numerous), and recent canopy dieback (0 to 6 scale, below). Decay impact ratings (none, low, moderate, high) were made by assessing the probability that current apparent decay levels would negatively impact tree health or survival. The assessment of decay impact was based on the type(s) of decay present, location of decay within the tree, and the estimated extent of decay as rated by a trained observer. Additional details of the tree health rating methods are presented in Swiecki and Bernhardt (2001).

Stem water potential determinations

In September of each year (2000 to 2004), we collected midday stem water potential (SWP) readings on the center tree in each plot during the peak midday period (about 1300 to 1530 PDT). SWP methods followed those outlined by Shackel (2000) and have been previously reported (Swiecki and Bernhardt 2002a). In addition, starting in 2001, we took SWP readings on plot trees other than the plot center tree (extra SWP trees) in 45 of the 150 plots for comparative purposes. One extra SWP tree was used in 44 plots; the remaining plot had two.

Additional tree and plot variables

Plot center trees and the 47 extra SWP trees were also rated for origin class (seed or coppice), stem count, DBH, and amount of crown exposure to overhead sunlight (0 to 6 scale, below). Plot variables recorded included plot slope and aspect, total basal area, tree counts by species, plot canopy cover, woody understory cover, cover of selected tree species and poison oak (*Toxicodendron diversilobum*). We also recorded the disease status of all other coast live oak, California black oak, and tanoak trees in the plot with respect to *P. ramorum* and other pathogens, and counts of regeneration of these three species. Coast live oak, black oak and tanoak trees other than the plot center tree are collectively referred to as plot trees in this paper.

In 2003, we collected data on physical characteristics of the bark of coast live oak plot center and extra SWP trees at all locations except 9 and 12, which did not include coast live oaks. We used a variety of descriptors to rate bark morphology of the lower bole. Bark characteristics rated included the abundance (none, trace, low to moderate, moderate to dense) and location of epiphytic lichens and mosses, various surface bark morphologies (striate, checkered, smooth, furrowed, irregular), the presence of shallow, medium, or deep bark fissures, the presence of deep bark cracks, and the presence of non-weathered, brown bark in the center of furrows or fissures. We rated the relative abundance of this last characteristic using the 0 to 6 scale in the 2004 evaluations.

We used the following arcsine-transformed percentage scale for most ocular estimates of percentages: 0 = not seen, 1= less than 2.5 percent, 2 = 2.5 percent to 19 percent, 3 = 20 percent to 49 percent, 4 = 50 percent to 79 percent, 5 = 80 percent to 97.4 percent, 6 = more than 97.5 percent.

Bark thickness

Bark thickness was measured using a needle-type bark probe, which functioned in the same way as the one described by Gill and others (1982). The bark probe measures the distance between the outer bark surface and the wood using a blunt-ended 1.9 mm diameter steel probe that is pushed by hand pressure through the bark layers. The probe tip does not penetrate the wood, so the depth of penetration, measured to the nearest millimeter, indicates total bark thickness at the point of insertion. All bark depth measurements were made in "average" bark depth areas, avoiding atypically high or low spots.

To determine the optimal height to measure bark thickness, we conducted a small pilot study using 19 trees located beyond the edges of the study plots. For these trees, we measured bark thickness at each of the cardinal compass directions at 0.5, 1, 1.5, and 2 m above the ground surface. The four readings at each height were averaged to give a mean bark thickness reading for each tree at each height.

Based on the results from the pilot study (discussed in Results below), we selected 1 m as the height for measuring bark thickness for other trees in the study. To minimize possible effects associated with bark probing on study trees, bark thickness data in 2003 was measured only on dead trees in the plots and extra SWP trees. The status of the bark at each point of measurement (live, dead but moist, dead and dry) was recorded. For dead trees and dead areas of living trees, bark was chipped open so that its thickness could be measured while viewing the bark in a radial or cross section. This prevented errors associated with gaps between wood and dead bark or penetration of the probe into decayed wood. On live trees, the bark area to be probed was sprayed with 70 percent isopropanol prior to insertion of the bark probe, which was cleaned with 70 percent isopropanol before each probe. After determining that bark probing of live trees did not initiate disease or cause significant damage, we expanded bark probe measurements to include all remaining plot center trees in fall 2004.

To adjust thickness measurements to account for shrinkage due to drying, we conducted a separate study of radial bark shrinkage upon drying. Bark thickness measurements were made using the bark probe on 35 fresh sections of coast live oak. Stems were then allowed to air dry and bark thickness was remeasured. For 22 samples, we also obtained measurements for partially dried bark (i.e., dead but moist status). The average percent shrinkage in thickness for all samples was 22.5 percent for fresh to dry bark and 12 percent for fresh to dead but moist bark. These shrinkage values were used to adjust individual bark thickness measurements of

dead/dry or dead/moist bark samples to the estimated fresh bark thickness prior to averaging and analysis.

Statistical analyses

We used JMP® statistical software (SAS Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $P \leq 0.05$. The likelihood ratio chi square statistic was used to test the significance of difference of proportions in 2 × 2 contingency tables. Effects of year and tree variables on SWP in 2000 to 2004 were tested using repeated measures analysis of variance. We used linear regression to test for associations between continuous outcomes (e.g., SWP) and continuous or categorical predictor variables. The nonparametric Spearman test was used to test for correlations between pairs of categorical variables (e.g., those using the 0 to 6 rating scale). We also used analysis of variance (F-tests) or t-tests to test whether mean levels of continuous variables differed between groups such as cases and controls.

We developed logistic regression models to examine the effects of factors on the binary disease outcome (plot center tree is diseased, i.e., a case) or a binary disease progress outcome (disease progress observed). We screened possible predictor variables using univariate logistic regressions, examined correlations between predictor variables, and tested whether models were strongly influenced by outlying observations by comparing models with and without extreme outliers. We also used recursive partitioning (also known as regression trees, CART™, etc.) to develop preliminary models, investigate interactions between predictors, and determine optimum thresholds for creating binary variables. This procedure splits data in a dichotomous fashion according to a relationship between the predictor and outcome values, creating a tree of partitions. Each partition is chosen to maximize the difference in the responses between the two branches of the split.

We developed multivariate models using a stepwise procedure. Factors were generally considered for entry into the multivariate models if odds ratios from univariate models were significant at $P \leq 0.10$. The significance level of each factor reported in the final models should be interpreted as if it were the last factor added to the model. We also calculated Akaike's information criterion (AIC) to compare the fit of alternative models. For models constructed for a given data set, smaller AIC values indicate better model fit.

Results

Symptom development and disease progress 2000 to 2004

Overall disease incidence and mortality

Among 122 live tanoaks that were asymptomatic in 2000, 21.3 percent showed disease symptoms by September 2004. These presumably represent both new infections and trees with latent infections that developed visible symptoms during the observation interval. Among 473 live coast live oaks that were asymptomatic in 2000, 23 (4.9 percent) showed disease symptoms by 2004. This increase in disease incidence is significantly less than that seen in tanoak (likelihood ratio $p<0.0001$). More than 50 percent of the newly symptomatic coast live oaks first exhibited symptoms in 2003. In contrast, the largest yearly increases in the number of newly symptomatic tanoaks were observed in the 2001 and 2004 ratings.

Although newly symptomatic trees have been observed among both tanoak and coast live oak between 2000 and 2004, only tanoak shows a steady net increase in disease over this period (*fig.1*). Including recently-killed trees present at the start of the study in the base tree population, the overall *P. ramorum* canker incidence in tanoak has increased from 31 percent in 2000 to 43 percent in 2004 (*fig.1*). For coast live oak, overall *P. ramorum* canker incidence increased only from 23 percent in 2000 to 24 percent in 2004 (*fig.1*). In coast live oak, 16 of the 124 live coast live oak trees that had *P. ramorum* canker symptoms for at least the first two years of the study have been reclassified as asymptomatic due to a lack of apparent cankers in later ratings. Losses in symptomatic coast live oaks largely offset the gains due to new symptom development, leading to almost no net change of *P. ramorum* canker incidence in coast live oak over the period. In contrast, none of the tanoaks with *P. ramorum* symptoms in 2000 had become asymptomatic by 2004.

As seen in *fig. 1*, mortality due to *P. ramorum* has occurred at significantly higher rates in tanoak than in coast live oak (likelihood ratio $p<0.0001$). In addition, most mortality occurred among trees that were already symptomatic in 2000. Among live trees that had *P. ramorum* canker symptoms in 2000, 35 percent of tanoaks (n=34) and 26 percent of coast live oaks (n=124) had died by 2004. Among trees that were asymptomatic in 2000, 6 percent of tanoaks but only 0.4 percent of coast live oaks developed *P. ramorum* canker symptoms and died by 2004.

Futhermore, symptomatic tanoaks typically died more rapidly than coast live oaks. Among tanoaks with *P. ramorum* canker symptoms that were live in 2000 and had died by 2004 (n=21), 57 percent died within one year and 81 percent died within two years after symptoms were initially observed. In contrast, for coast live oaks (n=34), 26 percent died within one year and 62 percent died within two years after *P.*

ramorum symptoms were initially observed. These totals include trees that developed symptoms after 2000 as well as trees that were symptomatic in 2000, so some trees may have been symptomatic longer than the periods noted.

P. ramorum canker has been the main cause of mortality in tanoak study trees (*fig. 1*). Because most tanoak trees in the two tanoak study locations are relatively young and vigorous, only one tanoak has died due to agents other than *P. ramorum.* This was a 4 cm DBH root sprout originating from a tree which died and failed before the study started. The sprout died as the result of root disease associated with the failed stem. In contrast, canker rots and other wood decay fungi are relatively common in the coast live oak study locations, and these agents are associated with decline and death of numerous coast live oaks in the study (*fig. 1*).

In trees with multiple stems, each stem generally functions independently with respect to *P. ramorum* canker. Death of main stems generally occurs independently and is related to canker severity on each stem. We recorded symptom data for individual stems in plot center and extra SWP trees for coast live oaks and tanoaks. Of the 70 multistemmed trees in this subset, 14 of 56 coast live oaks and 10 of 14 tanoaks had symptoms of *P. ramorum* canker in at least one main stem (*fig. 2,* left). However, in a substantial percentage of these symptomatic trees (7 coast live oaks and 7 tanoaks), main stems with and without *P. ramorum* symptoms were present in the same tree. By 2004, there were more multistemmed trees with a mixture of both live stems and stems killed by *P. ramorum* than multistemmed trees in which all stems had been killed by *P. ramorum* (*fig. 2,* right).

Many trees killed by *P. ramorum* cankers produce basal sprouts that can persist for at least several years after the death of the top. In addition, some trees with *P. ramorum* cankers that have experienced bole failures while still alive have subsequently produced vigorous epicormic sprouts on the remaining portion of the bole. In 2004, 81 percent of tanoaks (33/39) and 43 percent of coast live oaks (22/51) with tops killed by *P. ramorum* cankers over the study still had live basal sprouts or epicormic sprouts arising after bole failure. The data on sprouting and disease in multistemmed trees support our general field observations that, in most trees with *P. ramorum* canker, the decline of the top is directly related to the presence of stem cankers and is not associated with decline due to pre-existing root disease.

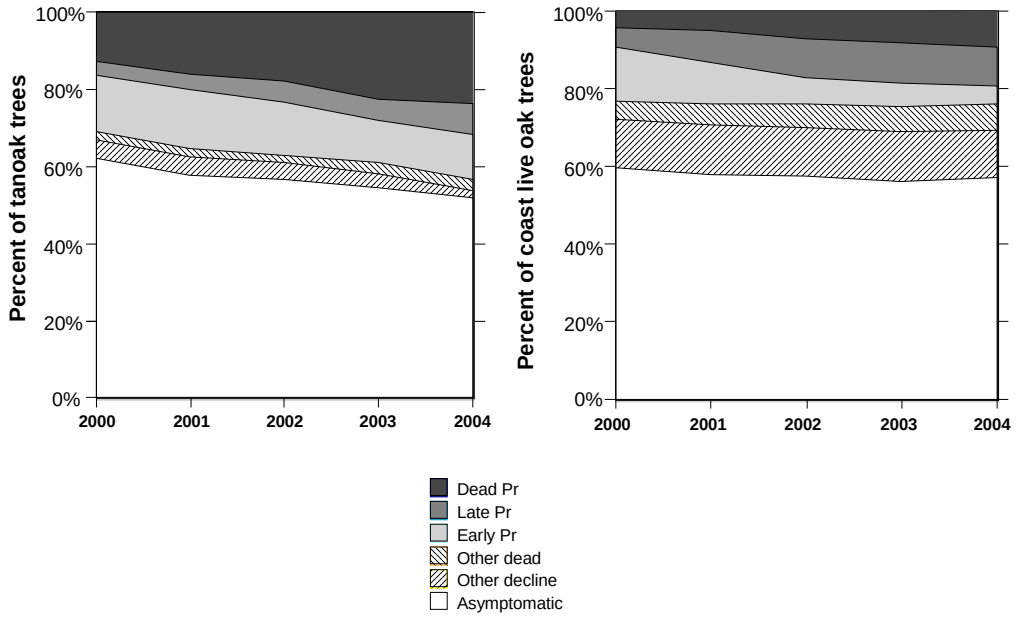

Figure 1— Changes in health of all tanoak (n=187) and coast live oak (n=655) study trees from September 2000 to September 2004. **Dead Pr** = tree dead as a result of *P. ramorum;* **Late Pr** = live trees with *P. ramorum* cankers plus beetle boring and /or *H. thouarsianum* fruiting bodies; **Early Pr** = live trees with *P. ramorum* cankers only; **Other dead** = tree dead due to agents other than *P. ramorum;* **Other decline**=tree in severe decline due to agents other than *P. ramorum;* **Asymptomatic**= no evident symptoms of *P. ramorum* infection or decline due to other agents.

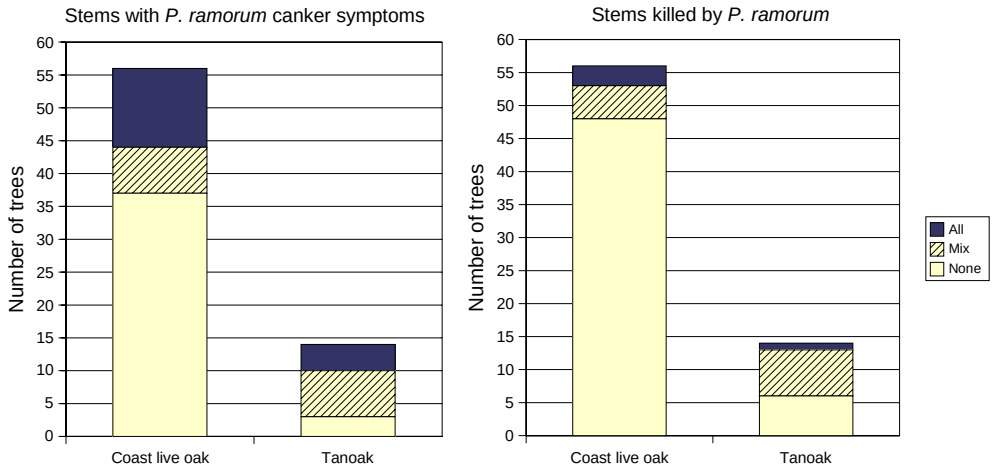

Figure 2 — Occurrence of *P. ramorum* canker symptoms (left) or *P. ramorum*-related mortality (right) among individual main stems of multistemmed coast live oak (n=56) and tanoak (n=14) in September 2004. **All** = all main stems symptomatic or dead; **Mix** = mixture of asymptomatic and symptomatic (left) or live and dead (right) stems; **None** = no stems symptomatic or dead.

Symptom development

In coast live oak and tanoak trees that were killed by *P. ramorum* canker, early symptoms typically consisted of bleeding bark cankers, but cankers did not necessarily bleed in all years. Recent bleeding (within the previous 6 to 12 months) was seen in 26 percent of the live tanoaks with *P. ramorum* symptoms each year from 2002 through 2004. In comparison, recent bleeding was seen in 39 percent of the live, symptomatic coast live oaks in 2002 and 2004, and in 54 percent of the live, symptomatic coast live oaks in 2003.

Over time, most *P. ramorum* cankers expanded in both radial and longitudinal directions. The number of apparently separate cankers tended to decrease over time, either because adjacent cankers merged or because the true extent of cankers became more obvious over time. We rated the percent of the bole circumference girdled by *P. ramorum* cankers in the plot center and extra SWP trees at each annual evaluation. For both tanoak (n=17) and coast live oak (n=52) trees with *P. ramorum* canker symptoms, the mean 2004 *P. ramorum* girdling rank of trees with only early disease symptoms was significantly less than the girdling rank of trees that had progressed to late disease symptoms or died (*table 2*). All of the trees that had died were at least 50 percent girdled by *P. ramorum* cankers.

Table 2— *Percent of total main stem circumference affected by* P. ramorum *cankers in tanoaks (n=17) and coast live oaks (n=52) by disease stage in 2004*

	Mean (sd) *P. ramorum* canker girdling rating[1]		
Species	**Early symptoms**	**Late symptoms**	**Trees killed by *P. ramorum***
Tanoak	2.60 (0.55) a[2]	4.44 (0.73) b	5.00 (0) b
coast live oak	2.36 (1.36) a	3.97 (1.17) b	4.67 (1.07) b

[1] Percent of main stem circumference girdled by *P. ramorum* cankers estimated using 0 to 6 scale (see methods).

[2] Means followed by different letters are significantly different ($p<0.05$) according to Tukey-Kramer HSD test.

We collected data on the apparent height of the upper and lower margins of *P. ramorum* cankers in coast live oak plot center and extra SWP trees to help determine an appropriate height range for studying the relationships between bark characteristics and disease. The lower margins of all measured cankers (n=60) occurred within 1.3 m of the soil grade, with most (80 percent) occurring within the lower 0.4 m of the bole. The mean height for all lower canker margins was 27 cm (s.d. 37). The height of the upper canker margin of the highest cankers was quite variable, with an overall mean of 1.11 m (s.d. 56) above the soil grade and a range from 0.1 to 2.2 m above soil level. Only about 25 percent of the upper canker margins were higher than 1.5 m.

Late symptoms of *P. ramorum*-related decline include ambrosia beetle boring and/or stromata of *H. thouarsianum* that develop on larger cankers and may extend well beyond the cankers around and up the stem. All of the coast live oak study trees with *P. ramorum* symptoms that died between 2000 and 2004 had beetle boring and/or stromata of *H. thouarsianum* in the year prior to mortality and almost all trees had both secondary agents visible in the year that death was recorded (*table 3*). However, small diameter tanoaks did not consistently exhibit beetle boring and/or *H. thouarsianum* stromata (late symptoms) before being killed by *P. ramorum* cankers (*table 3*). The trees that had no evident beetle boring or *H. thouarsianum* stromata in the year that they died ranged from 4.5 to 17 cm DBH. Hence, although many smaller tanoaks were apparently killed by *P. ramorum* cankers alone, secondary agents were consistently present in coast live oaks with *P. ramorum* cankers that died.

Table 3— *Number of tanoaks and coast live oaks with P. ramorum cankers that also had beetle boring or H. thouarsianum stromata in the year that tree mortality was recorded*

Species	beetle boring	*H. thouarsianum* stromata	no beetles or *H. thouarsianum*	Total trees[1]
tanoak	7	4	7	15
coast live oak	28	29	0	29

[1] Data set includes plot center trees and extra SWP trees that were live in 2000 and died by 2004 and other plot trees that were live in 2001 and died by 2004.

Many *P. ramorum*-infected coast live oaks experienced major failures before the top had died. Between 2000 and 2004, we observed 56 failures (bole failures or branch failures ≥20 cm diameter) among 153 coast live oaks with *P. ramorum* symptoms. This 36 percent failure rate in *P. ramorum*-infected trees is significantly greater (likelihood ratio $p<0.0001$) than the 4 percent failure rate seen over this period among 484 coast live oaks without *P. ramorum* symptoms.

Limited symptom development

Although symptom development commonly proceeds as described above, some trees have shown a lack of symptom progress beyond the early or late symptom stage over the course of the study. Overall, 47 percent of the tanoaks and 58 percent of the coast live oaks with early or late *P. ramorum* canker symptoms in 2000 advanced to a more advanced symptom class (late or dead, respectively) by 2004.

Among the smaller set of trees for which we have ratings of the percent of the main stem girdled by *P. ramorum* cankers, three of 17 (18 percent) symptomatic tanoaks

and 16 of 60 (27 percent) coast live oaks showed no apparent disease progress between 2000 and 2004 based on either canker girdling rating or symptom class. Cankers in most of these trees exhibited little or no bleeding and appeared to be inactive. These counts include all trees that were rated as symptomatic at least three of the five years, and excludes 16 coast live oaks that had been rated as having *P. ramorum* canker symptoms for at least two years and were reclassified as asymptomatic by 2004. These trees initially had small cankers that had subsequently been inactive for several years.

Over the past several years, we have also noted the production of apparently healthy callus at the edge of *P. ramorum* cankers in coast live oak and tanoak trees (*fig.3*), especially in trees that have shown little or no symptom progress from year to year. Callus may either partially or completely encircle cankers. Callus development was noted in the detailed disease ratings for three of 17 live tanoaks and 22 of 52 live coast live oaks with *P. ramorum* cankers in 2004.

Figure 3— Callus development around inactive *P. ramorum* cankers in coast live oak (left) and tanoak (right). Arrows indicate callus edge.

Many trees with late *P. ramorum* canker symptoms have developed elevated levels of diffuse canopy dieback during the study (*fig. 4*). This gradual dieback and canopy thinning occurs in trees with extensive cankers that survive for at least several years. It is distinct from the rapid dieback of the entire canopy ("sudden death") seen in trees that die within one to two years from the appearance of stem cankers. Among *P. ramorum*-infected trees that were still alive in 2004, canopy dieback rating was positively correlated with the *P. ramorum* stem girdling rating (Spearman R=0.555,

p=0.034, n=14 for tanoak; Spearman R= 0.367, p=0.020, n=40 for coast live oak). Diffuse canopy dieback also increases over time in trees that are in severe decline due to other mortality agents, such as canker rot fungi (*fig. 4*).

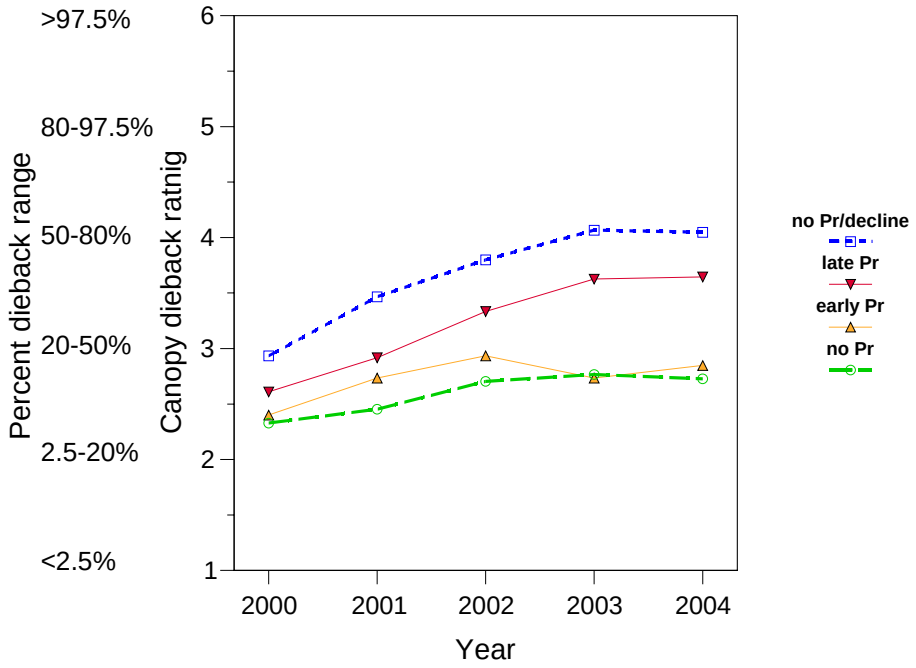

Figure 4— Canopy dieback ratings from September 2000 to September 2004 in coast live oak that were still living in 2004; **no Pr/decline**=trees in severe decline due to agents other than *P. ramorum*, **late Pr**=live trees with *P. ramorum* cankers plus beetle boring and /or *H. thouarsianum* fruiting bodies; **early Pr**=trees with *P. ramorum* cankers only, **no Pr**=non-declining trees with no evident symptoms of *P. ramorum* infection.

Stem water potentials of coast live oaks and tanoaks

In a repeated measures analysis of variance of SWP, the effects of year and species were highly significant (p<0.0001), but the interaction between these factors was not (p=0.071). Tanoaks consistently had higher average SWP readings than coast live oaks (*fig. 5*). Overall mean SWP fluctuated from year to year, apparently in response to the level of precipitation in the previous year (*fig. 5*). For coast live oak, the study location average SWP was positively, but not strongly, correlated with the rainfall for the location and year (adjusted R^2=0.080, p=0.026, n=10). Our rainfall data were from nearby weather stations rather than from the actual study sites, which may have weakened the apparent correlation.

Stem water potentials (SWP) measured in September of 2000, 2001, 2002, 2003 and 2004 were highly correlated from year to year for individual coast live oak and tanoak trees. A correlation matrix analysis showed that SWP readings from individual trees in different years were significantly correlated (p<0.0001) in all

combinations, with adjusted R^2 values ranging from 0.71 to 0.85. Although mean SWP varied from year to year, we found that the SWP of most trees shifted up or down by an amount that approximated the overall mean year to year difference. After removing the overall year to year differences, the SWP of individual trees were quite consistent from year to year. This suggests that relative SWP levels measured over the study period were likely to be similar to those that existed prior to the start of the study, i.e., during the period when most trees were initially infected.

To determine whether the SWP of the plot center tree (case or control) was representative of the water status of the plot as a whole, we compared SWP readings for 2001 through 2004 from plot center trees with the extra SWP trees present in 45 of the 150 plots. Readings between pairs of trees from the same plot (n=45) were significantly correlated in all four years (adjusted R^2 varied between 0.519 and 0.784 in the different years). This suggests that much of the variation in SWP is related to the available soil moisture level within the plot, which is influenced by local factors including soil type and depth, slope, aspect, and vegetative cover. Hence, SWP of any tree in a plot (center tree or a different tree) provides an indication of tree water stress levels within the plot as a whole.

Because year to year variations in SWP tend to obscure directional changes in SWP over time, we used repeated measures analysis of variance to test whether SWP was influenced by disease progress. For this analysis, we compared SWP of coast live oaks that were asymptomatic in 2000 through 2004 (n=107) to trees that had progressed from early to late *P. ramorum* symptoms between 2000 and 2004 (n=22). In this analysis, only the effect of time was significant ($p<0.0001$); neither disease status (asymptomatic vs. early to late) nor the interaction between disease status and time were significant. The same overall results are obtained if all live trees with *P. ramorum* canker in 2004 (n=36) are compared with asymptomatic trees. These results indicate that year to year changes in SWP were not affected by disease status, i.e., SWP in trees with *P. ramorum* did not change directionally over time.

Although the year-adjusted SWP of most trees with *P. ramorum* canker did not change over time, a few trees did show SWP changes. In several coast live oaks with late *P. ramorum* symptoms that were in the process of turning brown due to stem girdling, SWP readings were generally at least 1 MPa lower than previous readings, a difference well in excess of the overall annual change. In contrast, SWP readings on root sprouts of trees killed by *P. ramorum* were typically similar to or higher than readings made on the same trees before death of the top. This indicates that at least some portion of the root system continued to function after the death of the canopy in these trees.

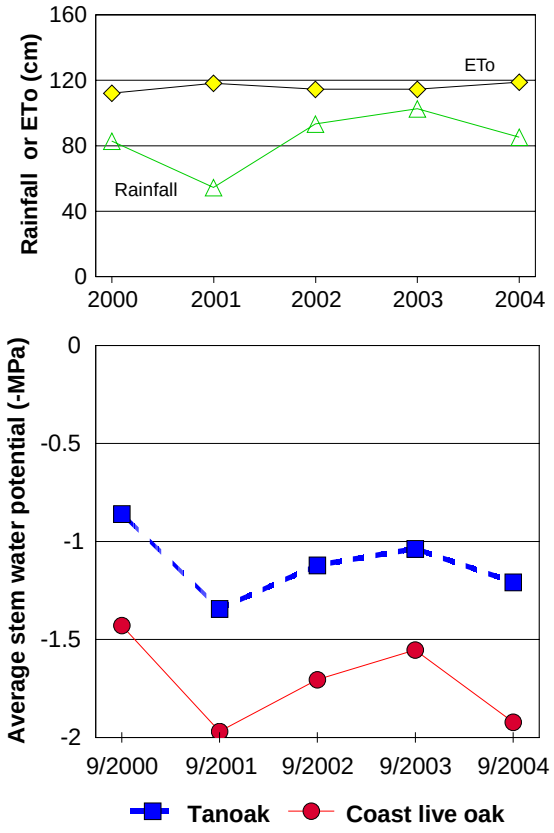

Figure 5— Average stem water potential for coast live oaks and tanoaks (bottom graph) across all locations and years compared with potential evapotranspirtation (ETo) and average seasonal rainfall (top graph); ETo is for September of previous year through August of listed year from the California Irrigation Management Information System (CIMIS) station in Santa Rosa; rainfall is the mean for all study locations calculated from the nearest weather station data for each location.

Bark characteristics and disease

Bark thickness

Initial bark thickness measurements were made at multiple heights (0.5, 1, 1.5, and 2 m) and the four cardinal directions on 19 healthy coast live oaks located beyond plot edges. We used analysis of variance to test whether bark thickness varied with cardinal direction or height above ground. This analysis showed no effect of cardinal direction but a significant linear decrease in bark thickness over the range of 0.5 to 2 m above grade ($p<0.0001$). For this sample, average bark thickness at 2 m (1.92 cm) was 70 percent of the mean bark thickness at 0.5 m (2.75 cm).

Based on the bark thickness data from these initial measurements and data on the vertical distribution of stem cankers (above), we selected 1 m as the standard sampling height for bark measurements in a sample of plot trees. Bark at this height was near its maximum thickness and measurements could be performed more readily

than at the lower (0.5 m height). Furthermore, most *P. ramorum* cankers on coast live oak study trees were found near or below this height.

Overall, the mean adjusted bark thickness of trees with *P. ramorum* canker symptoms in 2004 was significantly greater than that of the asymptomatic trees (t-test $p<0.0001$, *fig. 6*). Furthermore, mean bark thickness was greatest among trees that had already died and lowest among trees that still had only early symptoms of disease in 2004. Trees with late disease symptoms in 2004 had intermediate bark thickness levels (*fig. 6*). Given that most of these trees were symptomatic in 2000, this suggests that symptoms progressed more rapidly in trees with thicker bark. Bark thickness was also positively correlated with other indicators of disease severity, including the number of bleeding cankers in 2001 (adjusted $R^2=0.101$, $p=0.008$) and the rating of the percentage of the bole girdled by *P. ramorum* cankers in 2001 (adjusted $R^2=0.098$, $p=0.009$).

Among all (symptomatic and asymptomatic) coast live oaks, bark thickness increased in a nonlinear fashion with increasing stem diameter (*fig. 7*), with an upper plateau near 5.5 cm. As seen in *fig. 7*, bark thickness can vary by 2 cm or more for trees with a given stem DBH. Bark thickness increased as the tree's sky exposed canopy rating increased (adjusted $R^2=0.17$, $p<0.0001$ for quadratic regression line), suggesting that bark thickness is generally greater in more dominant trees. In addition, among coast live oaks without *P. ramorum* canker symptoms, trees that were severely declining due to other agents or factors had significantly thinner bark (mean 2.2 cm, n=23) than non-declining trees (mean 2.7 cm, n=113; t-test $p<0.0001$). Overall, these data suggest that bark thickness was generally greater in more vigorous, faster-growing coast live oaks. These dominant, fast-growing coast live oaks also had a higher risk of developing *P. ramorum* canker symptoms than suppressed, slow-growing oaks in the same stands.

Bark surface characteristics

Most bark characteristics, including the abundance and location of epiphytic lichens and mosses, various bark morphologies (striate, checkered, smooth, furrowed, irregular), and the presence of bark fissures of various depths occurred at nearly equal frequencies in symptomatic and asymptomatic trees and were thus not predictors of disease status. However, one bark characteristic was a significant predictor of the case (disease) outcome in both single variable and multivariate models. This was the presence of non-weathered, brown bark in the center of furrows or fissures that resulted from recent bark expansion (*fig. 8*).

To further explore this factor, we estimated the proportion of bark fissures on the lower bole that contained unweathered brown bark using the 0 to 6 scale. Ratings were made on plot center trees in 2004. This quantitative brown fissures variable was a significant predictor of the case outcome in both single and multivariate models, as noted below. However, brown fissures were not significantly correlated with variables related to disease progress, including the amount of *P. ramorum* girdling and progress in disease symptoms between 2000 and 2004. This suggests that this bark characteristic may be related to risk of infection, but not to later canker expansion.

Average brown fissure ratings were significantly lower among severely declining trees without *P. ramorum* symptoms (mean 0.30, n=20) than among relatively healthy (non-declining) asymptomatic trees (mean 2.2, n=96; t-test $p<0.0001$). Brown fissure ratings also declined as decay impact ratings increased. Furthermore, brown fissure rating was positively correlated with sky exposed canopy rating (adjusted $R^2=0.31$, $p<0.0001$, n=123), and negatively correlated with plot canopy cover (adjusted $R^2=0.12$, $p<0.0001$, n=123). These correlations suggest that brown fissures are more common in trees that are likely to have at least moderate growth rates. However, brown fissure ratings were not correlated with either stem diameter or bark thickness. Hence, although brown fissures and bark thickness may both be related to tree vigor or growth rate, they appear to reflect different ways that bark morphology can be affected by growing conditions.

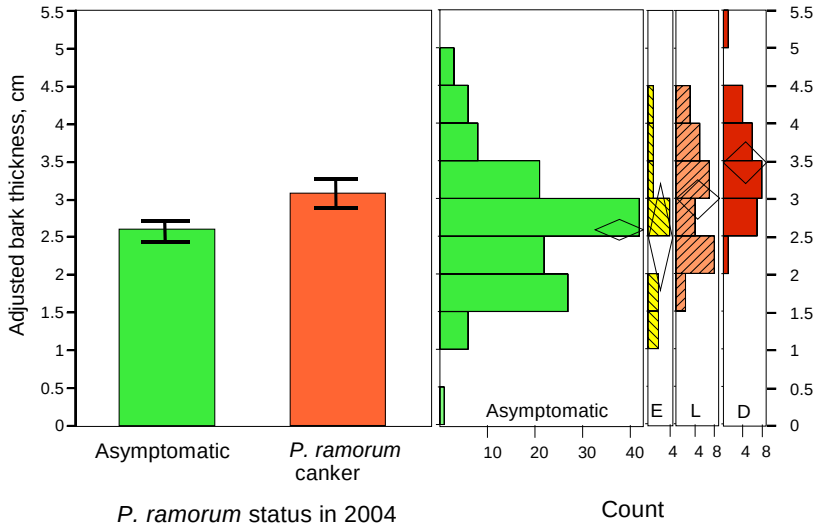

Figure 6—Relationship between coast live oak bark thickness and *P. ramorum* disease status in 2004; bark thickness readings in dead bark samples were adjusted to account for shrinkage due to drying; error bars (left graph) and outer points of means diamonds (histograms, right) denote 95 percent confidence intervals of the means; *P. ramorum* symptoms in histograms are E=early (bleeding cankers only), L=late (cankers + beetles and/or *H. thouarsianum*), D=dead; n=203

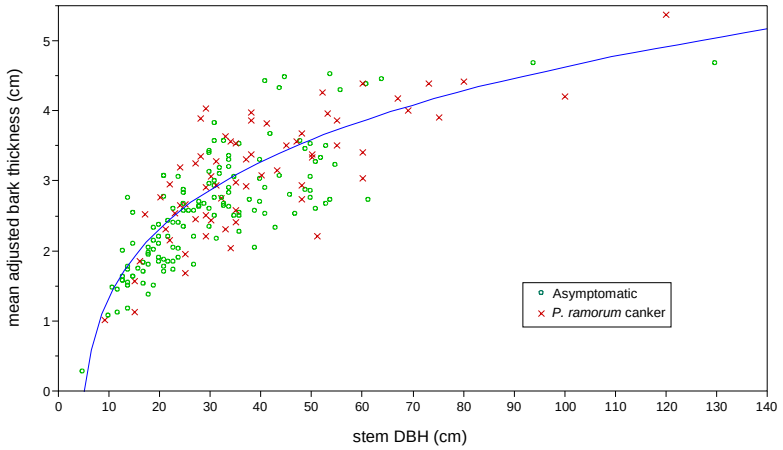

Figure 7— Relationship between bark thickness at 1 m height and stem diameter (at 1.37 m height, DBH) for coast live oaks with or without symptoms of *P. ramorum* canker; fitted curve is $e^{(\text{adjusted bark thickness})} = -1.52 + 0.471(\text{DBH}) + 0.00573(\text{DBH})^2$, adjusted R^2=0.62, p<0.0001, n=202

Figure 8 - Images of coast live oak bark on the left have unweathered brown bark within bark fissures, presumably resulting from recent bark expansion. Unweathered brown areas are lacking in bark fissures shown on the right.

Disease risk models for coast live oak

We used recursive partition models and logistic regression to model the risk of *P. ramorum* infection in plot center trees. The outcome variable for these analyses included all plot center trees that had expressed likely *P. ramorum* canker symptoms through September 2004, including some initially symptomatic trees in which disease had apparently become inactive. Including these non-progressing trees maximized our ability to detect factors that may be related to infection but not later disease progress. For these models, tree and plot variables that could be considered outcomes related to disease were not tested as explanatory variables. For instance, diffuse canopy dieback was not included as a predictor because our data (above) suggest that it is a consequence of *P. ramorum* canker.

We constructed recursive partition disease risk models using both plot center trees only (n=128) and a larger data set that included extra SWP trees (n=168). The two initial splits were the same for both data sets, but subsequent splits varied between the data sets. In both models, the best initial split of the data was based on the brown fissure rating (0 and 1 vs. ≥2). About 90 percent of the trees with brown fissure ratings of 1 or less were controls. Within the group with brown fissure ratings of 2 or more, the next split for both models was based on 2002 SWP. About 80 percent of the trees with 2002 SWP <-2 MPa (relatively high water stress) were controls. Other splits in the models were based on further division by 2002 SWP (disease risk higher with high SWP/low water stress), the number of overstory and understory California bay in the plot (disease risk higher with more bay), sky exposed canopy (disease risk higher with greater canopy exposure), and bark thickness (disease risk higher with thicker bark).

We also developed logistic regression models for the case outcome using only the plot center tree data set. Two optimized models with similar fit based on AIC are shown in *table 4*. The two models differ only in that the SWP variable is replaced by a binary bark thickness variable (bark thickness greater than 3.3 cm). The optimum split for the binary bark thickness variable was determined from recursive partition models. Bark thickness was not significantly correlated with SWP overall. However, SWP was somewhat higher (t-test p=0.08) in trees with bark thickness greater than 3.3 cm than in trees with thinner bark. SWP readings from all years could be substituted for each other in the model, with 2002 SWP being the most significant. We used 2001 SWP in the model because it was the earliest year that SWP was measured in both plot center and extra SWP trees.

Table 4— *Logistic regression models for* P. ramorum *canker disease risk in coast live oak*

	Model 1[1]		Model 2[1]	
Predictor variables	**P level[2]**	**Odds ratio (CI)[3]**	**P level[2]**	**Odds ratio (CI)[3]**
Brown bark fissures rating (0-6 scale)	<0.0001	53.28 (10.01-363.68)	<0.0001	64.27 (10.85-531.30)
California bay cover in plot (0-6 scale)	0.0285	8.21 (1.25-61.11)	0.0009	21.06 (3.35-162.43)
More than 2 stems	0.0029	8.27 (1.98-46.72)	0.0028	9.11 (2.06-53.46)
2001 SWP (MPa)	0.0372	19.28 (1.88-396.59)		
Bark thickness>3.3 cm			0.0128	3.87 (1.33-12.33)

[1] The Akaike Information Criterion (AIC) for models 1 and 2 are 123.56 and 127.76 respectively. Overall significance levels of for both models were p<0.0001
[2] Likelihood ratio test significance level
[3] Odds ratios and 95 percent confidence intervals; odds ratios greater than 1 indicate that a factor is positively associated with the case (disease) outcome

Predictors in the model included only one plot variable, California bay cover, which is related to local inoculum density. Disease risk increases as bay cover in the plot increases, presumably because the amount of inoculum present in the plot also increases with bay cover. Other predictors are tree variables that may be related to host susceptibility. SWP can be considered both a tree and plot factor because SWP levels within a plot are correlated as noted above. Wetter conditions within plots (indicated by higher SWP levels) may favor host susceptibility, inoculum production, and the duration of suitable infection periods.

To validate the model, we used the model to predict the disease outcome in the extra SWP trees, which were not used to develop the models. For this smaller data set (8 cases, 32 controls), model one correctly predicted 72 percent of the outcomes and model two correctly predicted 69 percent of the outcomes. The case outcome was strongly underpredicted. However, the bay cover variable is based on cover in the 8 m plot around the plot center tree. Actual bay cover in an 8 m radius plot around the extra SWP trees could differ somewhat from this value, possibly contributing to poorer prediction of the case outcome.

Disease progress models for coast live oak

We also developed models for coast live oak to determine whether any of the factors we rated were related to disease progress or resistance in trees that were initially infected (*table 5*). This data set includes 58 coast live oaks (plot center and extra

SWP trees) which were alive and had *P. ramorum* canker symptoms in 2000. Of these, 42 showed disease progress, as shown by canker expansion and/or progression to a later disease stage (e.g., early to late or dead).

Indicators of initial disease severity, including canker count in 2001 and *P. ramorum* canker girdling rating in 2000 or 2001 are highly significant predictors of disease progress (likelihood ratio $p<0.0001$; AIC 37.4, 46.5, 44.7, respectively). Canker count was a somewhat better predictor of disease progress than canker girdling rating. Trees with greater numbers of cankers and/or a higher girdling rating in 2000 or 2001 were more likely to show disease progress than trees that initially had few and/or small cankers. The mean canker rating in 2000 was significantly higher (t-test $p=0.031$) for those that showed disease progress than for those that did not (mean ratings 3.0 and 2.1, respectively). However, because almost all of these trees were symptomatic at the start of the study, larger and more cankers in 2000 and 2001 can also be interpreted to be the result of disease progress up to that point. Thus, although canker severity at a given point in time may be a good indicator of future disease progress, these variables tell us little about factors that might predispose trees to greater disease progress.

The best multivariate model that excludes the initial disease severity variables is shown in *table 5.* When initial disease severity variables are excluded, bark thickness is the best single predictor of the disease progress outcome (likelihood ratio $p<0.0075$, AIC 64.5). As shown in *fig. 6*, this factor is positively correlated with disease risk and the disease stage in 2004, which is a component of the disease progress outcome.

The other factors in the model are more difficult to interpret. Disease was more likely to progress in trees located in plots with no or low levels of madrone cover. Recursive partition models showed that disease progress occurred in 88 percent of the trees in plots without any madrone cover but only occurred in 52 percent of the trees in plots with madrone cover. Furthermore, the likelihood of disease progress decreased as madrone cover increased. However, plots with the highest levels of madrone cover (greater than 20 percent) were only found at three of the 10 coast live oak study locations (five, six, and seven), so this variable may be partially confounded with other location-specific factors. Within the study areas, madrone tends to occur on soils that appear to be more shallow and are often more rocky than soils that are dominated by other tree species. This may indicate that coast live oaks growing in more unfavorable sites are less likely to show disease progress. However, SWP levels did not vary with madrone cover in these plots, and SWP was not a significant predictor of disease progress. The third factor in the model, poison oak

cover at least 2.5 percent, is not significant in a single variable model. In the multivariate model, the higher level of poison oak cover is positively correlated with disease progress.

Table 5— *Logistic regression model for* P. ramorum *disease progress in coast live oaks*[1]

Predictor variables	P level[2]	Odds ratio (CI)[3]
Bark thickness	0.0457	17.97 (1.054-450.6)
Madrone cover	0.0236	0.051 (0.00304-0.671)
Poison oak cover >2.5 percent	0.0488	7.878 (1.010-184.9)

[1]Overall significance level for the models is p<0.0021, AIC=61

[2] Likelihood ratio test significance level

[3] Odds ratios and 95 percent confidence intervals; odds ratios greater than 1 indicate that a factor is positively associated with the case (disease progress) outcome

Discussion

Over the course of the study, levels of disease and mortality due to *P. ramorum* have been consistently higher in tanoak than in coast live oak. Compared with tanoak, coast live oak had lower initial levels of disease observed in 2000, fewer trees that developed new symptoms between 2000 and 2004, and more trees that became asymptomatic after showing some early disease symptoms. These results support the overall consensus that tanoak is more susceptible to *P. ramorum* canker than coast live oak. In addition, our data suggest that conditions favorable for new bole infections occur more regularly in the tanoak study sites than in the coast live oak sites. Although apparent bole infection rates in tanoak have increased fairly steadily throughout the study period, most of the apparently new coast live oak bole cankers developed between the 2002 and 2003 ratings. This interval had the first relatively wet spring of the study period. Davidson and others (2005) found that *P. ramorum* inoculum levels in rainwater sampled in a coast live oak woodland in Sonoma County were greatly elevated in spring 2003 relative to the two previous years.

After five years of observation, we have been able to document several different symptom progression patterns in trees with *P. ramorum* canker. The "sudden oak death" pattern presented in the original descriptions of the disease (Garbelotto and others 2001, Rizzo and others 2002) involves fairly rapid canker expansion followed by a rapid dieback of the entire canopy of affected stems. Presumably many of the trees that were dead at the time of the initial evaluation in 2000 had followed this

pattern. Since that time, up to about 81 percent of tanoaks and about 62 percent of coast live oaks that have died have followed this rapid decline pattern. However, especially in coast live oak, cankers caused by *P. ramorum* are frequently smaller and less aggressive. Trees with less aggressive cankers follow different disease patterns, including apparent symptom remission in some trees.

Most tanoaks (65 percent) and coast live oaks (74 percent) with *P. ramorum* canker symptoms in 2000 were still alive in 2004. In coast live oak, trees with extensive late-stage cankers commonly develop diffuse canopy dieback (*fig. 3*, Swiecki and Bernhardt 2002c, 2004) that can eventually lead to canopy thinning. Some slow-declining tanoak and coast live oaks have developed healthy callus tissue around the margins of old *P. ramorum* cankers that do not appear to be expanding. Callus development is evidence of a host resistance response, but it is not clear whether callus development actually limits canker expansion or whether it is simply represents the normal host wound closure response acting in areas where pathogen activity has ceased for other reasons.

In addition, cankers in some coast live oaks and tanoaks have not expanded or oozed in several successive years and appear to be inactive. In the most extreme case of arrested disease progress, *P. ramorum* canker symptoms appear to have gone into remission in at least 16 coast live oaks. Because *P. ramorum* canker was not confirmed by culturing all symptomatic trees at the start of the study, it is possible that some of these trees represent trees that were not actually infected and had bleeding due to other causes. However, disease has failed to progress in some trees in which *P. ramorum* has been confirmed. Furthermore, the strong correlation between disease progress and initial disease severity suggests that disease progress is commonly limited in trees that have only a few small cankers. In addition, *P. ramorum* is typically difficult to recover from cankers in the latter part of the dry season of the year, and from older, relatively inactive cankers (D. Rizzo, personal communication). This suggests that the activity of *P. ramorum* varies over time within infected hosts. If *P. ramorum* cankers in coast live oak are limited at an early stage of development due to a combination of host resistance and low initial levels of infection, affected trees may be able to recover more or less completely.

Given that *P. ramorum*-infected trees have consistently had higher average SWP levels (i.e., lower water stress) than asymptomatic trees, we conclude that water stress is not a significant predisposing factor for the development of *P. ramorum* canker in coast live oak. To the contrary, trees with chronically high levels of water stress in September have a lower risk of developing *P. ramorum* canker. Because *P. ramorum* canker occurs more commonly in trees with both relatively low levels of

water stress and high canopy exposure (Swiecki and Bernhardt 2001, 2002a,b,c) with high potential transpiration rates, it is also unlikely that these are trees that had any significant impairment of root function prior to infection.

Furthermore, in coast live oaks with *P. ramorum* canker, the decline of the tree's canopy is driven primarily by the girdling of the bole rather than loss of root function. In *P. ramorum*-affected trees that show gradual canopy decline, canopy dieback reduces leaf area and hence transpiration demand. Consequently, symptomatic trees show no decrease in SWP and sometimes develop higher SWP levels over time despite symptom progress. If root decay or dysfunction was the primary cause of top dieback, lower SWP readings should have been common in symptomatic trees, rather than being limited to the few trees that are in the final stages of collapse due to extensive sapwood decay.

Many *P. ramorum*-killed coast live oaks and tanoaks have also produced vigorous basal sprouts or epicormic sprouts on the lower portions of failed stems that have survived for several years. This is evidence that at least some portion of the root system survives in these trees. In addition, independent disease progress among different stems observed in multistemmed trees (***fig. 2***) and canker girdling ratings (***table 2***) provide further evidence that decline of *P. ramorum*-infected trees is directly related to the number and extent of stem cankers, and is not primarily related to root disease. Although root pathogens, e.g., *Armillaria mellea,* eventually affect some trees with late *P. ramorum* canker symptoms, we have seen very little evidence of root disease among any of the trees in this study. Hence, several independent lines of evidence indicate that decline and death of trees with *P. ramorum* canker symptoms are not associated with pre-existing root disease or overall tree decline related to root dysfunction, but are a direct consequence of girdling by *P. ramorum* cankers.

Despite its relatively large host range, *P. ramorum* causes bark cankers on the main stems of only a few species under field conditions. This suggests that tree species that develop main stem cankers have relatively unique bark characteristics that permit *P. ramorum* to infect and colonize living bark tissues on mature main stems. For coast live oak, bark thickness and unweathered brown tissue within bark fissures were the only bark characteristics we rated that were positively correlated with *P. ramorum* disease risk. The latter characteristic appears to represent relatively rapidly expanding regions of the outer bark. Slow growing trees, including highly suppressed understory trees and declining trees, have very few or no fissures showing this characteristic, and are also at low risk for developing *P. ramorum* canker (Swiecki and Bernhardt 2001, 2002a,b,c).

Bark expansion zones may represent sites that are more easily breached by *P. ramorum* zoospores. It is also possible that the outer periderm in these areas may be thin enough that substances might diffuse from these areas when the bark surface is wet. If *P. ramorum* zoospores were chemotactically attracted to these zones, greater aggregation of zoospores cysts might occur in these areas, leading to a greater likelihood of successful infection. Bark fissures may also remain wet longer than other areas of the bark, increasing the chance of successful infection. These areas of the bark deserve further attention in studies of the infection process in coast live oak. The correspondence between brown fissures and high-moisture bark channels imaged by MRI (Florance, these proceedings) should also be investigated.

Bark thickness is the first variable we have measured in coast live oak that appears to be correlated with both disease risk (*table 4*) and disease progress (*table 5, fig. 6*). Coast live oak bark is relatively thick and consists primarily of living tissue, with only a thin (a few mm) outer layer of dead periderm (rhytiderm) in most trees. Trees with thicker bark appear to be more likely to become infected by *P. ramorum* and, once infected, disease appears to progress faster in trees with thicker bark. These findings are consistent with field observations that *P. ramorum* cankers do not naturally occur in either small diameter coast live oaks or in the smaller-diameter upper stems of coast live oak.

Several lines of evidence from this study indicate that *P. ramorum* canker is most likely to affect relatively vigorous or fast-growing coast live oaks, and is not primarily a disease of stressed trees. As we have previously reported, coast live oaks with *P. ramorum* canker tend to have more dominant canopy positions (higher levels of sky-exposed canopy) and larger stem diameter, and are less likely to be declining due to other agents or factors including canker rot and overtopping (Swiecki and Bernhardt 2001, 2002a,b,c). In addition, *P. ramorum*-infected trees have consistently had higher average SWP levels (i.e., lower water stress) than asymptomatic trees. Increased bark thickness and the presence of unweathered bark in bark fissures are additional variables that correlated both with faster tree growth and greater risk of *P. ramorum* canker. Although we examined a sample of increment cores to investigate recent growth rates, several technical issues made this approach impractical (Swiecki and Bernhardt 2003).

Because many of the factors that are related to faster growth are correlated with each other, it is difficult to determine which, if any, actually play important roles in affecting disease risk. For example, dominant and /or larger trees may intercept more inoculum, bark expansion zones may constitute favorable infection courts, and thicker bark may provide a better substrate for *P. ramorum* growth. However,

additional investigations are necessary to determine whether these and/or other factors correlated with tree growth rate actually contribute to disease risk.

Although we have identified a number of variables that can serve as predictors of disease risk in this study (*table 4*, Swiecki and Bernhardt 2001, 2002a,b,c), we have found very few good predictors of disease progress in infected trees. This is in part due to the fact that the data set for disease progress analyses is much smaller than the disease risk data set. Overall, initial disease levels observed in 2000 or 2001 were the best predictors of later disease progress, although these predictors are not completely distinct from the outcome. However, the significance of the factors in the model presented in *table 5*, shows that some tree and plot variables may help to predict the risk of disease progress in infected trees. Long-term data from a wider data set, such as all plot trees in this study, could be used to develop more robust disease progress models for coast live oak. However, we do not currently have detailed data, including bark thickness, needed to test disease progress models for the entire plot tree population.

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