Phytophthora ramorum canker (sudden oak death) in coast live oak and tanoak: factors affecting disease risk, disease progression, and failure potential

2003-2004 Contract Year Annual Report

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TABLE OF CONTENTS

Table of Figures **3**
Acknowledgements **4**
Summary **5**
Introduction **6**
Methods **7**
Study site selection 7
Plot selection 8
Stem water potential measurements 9
Bark characteristics 9
Additional tree and plot variables 11
Statistical analyses 12
Results and Discussion **17**
Stem water potentials (SWP) of coast live oaks and tanoaks 17
Comparison of average stem water potentials 2000 - 2003 17
Disease status and SWP 19
Relationships between California bay and *P. ramorum* canker 21
Vertical distribution of stem cankers 22
Coast live oak bark characteristics and disease 23
Bark thickness characteristics 23
Bark thickness and P. ramorum canker 24
Bark morphology 26
Disease progress 28
Coast live oak 28
Tanoak 30
Failures in coast live oak study trees 31
Literature Cited **34**

Cover photo: Three coast live oak trees at Novato, CA. Left tree lacks *P. ramorum* symptoms. Middle tree has late symptoms of *P. ramorum* canker and shows diffuse canopy dieback. Right tree has been killed by *P. ramorum* canker and has several large branch failures.

TABLE OF FIGURES

Figure 1. Map showing locations of study areas in Marin, Napa, and Sonoma counties. 8

Figure 2. Bark probe used to measure bark thickness..... 11

Figure 3. Average stem water potential for coast live oak and tanoak subject trees across all locations and years. 17

Figure 4. Average vapor pressure deficit (KPa) calculated from temperature and relative humidity measurements..... 18

Figure 5. Average temperature recorded in September of each year during stem water potential measurements at tanoak and coast live oak study locations..... 18

Figure 6. Rainfall and potential evapotranspiration (ETo) data for all study locations. 19

Figure 7. Stem water potential of coast live oak in September of each year for coast live oak trees that had the same disease status in all 4 years..... 21

Figure 8. Frequency distribution of heights of upper and lower margins of stem cankers on study trees 23

Figure 9. Relationship between adjusted bark thickness in coast live oak and *P. ramorum* disease status in 2003. 25

Figure 10. 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. 26

Figure 11. Coast live oak bark 27

Figure 12. Changes in health of all coast live oak study trees from September 2000 to September 2003. 29

Figure 13. Change in average canopy dieback rating over time for coast live oak subject trees scored in different disease categories in 2003..... 29

Figure 14. Changes in health of all tanoak study trees from September 2000 to September 2003..... 30

Figure 15. Increase in mortality among tanoak and coast live oak study trees alive in September 2000 due to *P. ramorum* (Pr) and other agents (e.g., wood decay or root rot fungi). 31

Figure 16. Increase in failures among coast live oak study trees grouped by disease status in September 2000. 32

Figure 17. Failures occurring in study trees classified by the most recent disease rating prior to failure. 32

Figure 18. Failure types of coast live oak trees occurring between September 2000 and September 2003. 33

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SUMMARY

We have completed four 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 2003, we collected data on *P. ramorum* symptoms, tree condition, stem water potential, and various other factors in 150 circular plots (8 m radius) in areas where *P. ramorum* canker was prevalent in 2000. Each plot is centered around a case (symptomatic) or control (asymptomatic) subject tree. Plots were established at 10 locations in Marin County, and 1 location each in Sonoma and Napa Counties.

Since September 2000, the percentage of symptomatic coast live oak trees in the plots has increased from 22.9% to 24.4%. Two-thirds of this increase in disease incidence occurred between September 2002 and September 2003, following the first relatively wet spring of the study. Between 2000 and 2003, the incidence of *P. ramorum* canker in tanoak has increased from 33% to 39%. For tanoak, increases in disease incidence were approximately equal in each observation interval, about a 2% increase in disease incidence per year.

Mortality due to *P. ramorum* among all monitored coast live oak increased from 3% to 8% between 2000 and 2003. Over the same period, *P. ramorum*-related mortality in all monitored tanoak has increased from 12% to 22.5% in 2003.

Between September 2000 and September 2003, substantial failures occurred in 32% of coast live oaks with *P. ramorum* symptoms but only 2% of coast live oaks lacking *P. ramorum* symptoms. Over this time interval, 73% of trees that were dead as a result *P. ramorum* canker in 2000 have failed, and 65% of the trees with late *P. ramorum* symptoms in 2000 (cankers with beetle boring and/or *Hypoxylon thouarsianum*) had failed. In contrast, only 10.5% of the coast live oaks with early *P. ramorum* symptoms (bleeding cankers only) in 2000 had failed by 2003 and less than 1% of healthy asymptomatic trees failed over this period. Bole failures were most common overall (58% of all failures) and root failures were the least common failure type (1.7% of the failures).

Because *P. ramorum* primarily causes a bark canker in coast live oak, we initiated a study to determine whether observable bark characteristics are related to *P. ramorum* canker occurrence or progress. In 2003, we evaluated both bark thickness at 1 m height and a variety of morphological bark characteristics in a subset of study trees. In this sample, we found that *P. ramorum* canker was more likely to occur in trees with greater bark thickness. We also observed that coast live oak bark thickness increases in a nonlinear manner as tree diameter increases, which suggests that relationships between tree diameter and disease could be related to bark thickness. Only one of the bark morphological characteristics we assessed, the presence of unweathered bark in bark furrows, was positively correlated with disease. This characteristic seems to be associated with faster rates of tree radial growth, and is consistent with other analyses indicating that faster-growing coast live oaks have a greater risk of developing *P. ramorum* canker than slow-growing trees.

Based on four successive years of stem water potential measurements on the same set of trees, it appears that SWP values measured in September are mainly influenced by rainfall over the three preceding rainy seasons. SWP readings for individual trees in all three years are also highly correlated.

SWP readings indicate that water stress is not a significant predisposing factor for the development of *P. ramorum* canker in coast live oak. Most coast live oaks with *P. ramorum* canker symptoms have maintained relatively high SWP levels and do not show progressive increases in water stress as disease develops. We hypothesize that SWP levels in many of these trees with advanced symptoms of disease remain high because of the progressive diffuse canopy dieback that develops in trees with advanced *P. ramorum* canker symptoms. Leaf area loss resulting from branch dieback reduces evapotranspiration while roots continue to function, which allows trees to maintain high SWP levels.

INTRODUCTION

In the summer of 2000, *Phytophthora ramorum* (then an unnamed new species) was identified as the cause of bark cankers on the main stems of tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*), and California black oak (*Q. kelloggii*) (Garbelotto and others 2001). The disease was dubbed "sudden oak death" (SOD) because mortality of infected trees was the first widely recognized symptom when the disease was initially observed in the mid 1990's. Subsequent studies have shown that early symptoms of the disease consist of bark cankers which typically ooze or bleed a reddish to dark brown exudate (Rizzo and others 2002b). 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). These agents also attack declining trees or branches that are not infected with *P. ramorum*.

Stem cankers caused by *P. ramorum* appear to be limited to aerial portions of the plant. *P. ramorum* cankers are typically found on the lower bole of affected trees, but seldom extend more than a few centimeters below the soil surface (Rizzo and others 2002b). To date, *P. ramorum* has not been associated with root decay of oak or tanoak. This characteristic differentiates *P. ramorum* cankers from those caused by other common *Phytophthora* species such as *P. cinnamomi* (Garbelotto and others 2001, Rizzo and others 2002a, 2002b). The disease situation is complicated by the fact that at least two other previously unrecognized species of *Phytophthora* can cause symptoms similar to those caused by *P. ramorum* and have overlapping host and geographic ranges. Cankers caused by *P. nemorosa* (Hansen and others 2003) and *P. pseudosyringae* (D. Rizzo, personal communication) appear to be somewhat less common and may be less lethal than those caused by *P. ramorum*, but research on these additional species is still ongoing.

At the time this study was initiated in August 2000, very little was known about the epidemiology of this disease. We considered water stress a possible risk factor for disease development because affected trees are commonly found in highly competitive situations. Water stress occurring either before or after infection has been shown to increase the susceptibility of various plants to *Phytophthora* spp. (Sinclair and others 1987) and is also a predisposing factor for *Hypoxylon* infection (Sinclair and others 1987) and beetle attack.

To examine the role of water stress and other factors on the development of *Phytophthora* bole cankers, we conducted a case-control study in areas where the disease syndrome is common and *P. ramorum* had previously been isolated from infected trees. In a case-control study, a group of subjects that exhibit a particular outcome (e.g., disease), referred to as the case group, is compared with a second group of subjects that do not exhibit the outcome, referred to as the control group. Factors preceding the outcome are then compared between groups and the factor-outcome association is assessed statistically. Evaluated factors may increase, decrease, or have no effect upon the risk of the outcome under study. This study design is descriptive and quantitative, but only allows associations to be explored. Although direct causality cannot be proven from a case-control study, possible cause and effect relationships can be identified for further study. The case-control study design allows for a rapid assessment of potential risk factors. The magnitude of the association between risk factors and an outcome, such as disease, can also be assessed. However, the models cannot be used to predict disease levels in a population.

In this study, we are evaluating factors associated with *Phytophthora* canker risk in coast live oak. We also collected a limited amount of information on tanoak for comparative purposes. We are assessing whether water stress and various other tree and stand factors are risk factors for the early phase of the disease, i.e., the bleeding bark cankers that are associated with *Phytophthora* infections. Disease risk models based on results from the first three years of this project have been reported (Swiecki and Bernhardt 2001a, 2002a, 2002b, 2003b). We have continued to refine these disease risk models by testing additional predictor variables and reclassifying trees that have developed symptoms since the first year of the study.

As the study has progressed, we have increasingly focused on disease progress, mortality, and tree failure in the study population. This allows us to investigate risk factors that are associated with disease progress, mortality, and failure in infected trees. We are also able to document different patterns of disease progress that may be correlated with host resistance. This report presents results from the fourth year of observations from this ongoing study.

METHODS

Study site selection

During September 2000, we established plots at 12 study locations (Table 1, Figure 1). Study sites were selected on the basis of appropriate vegetation type (adequate representation of coast live oak or tanoak), the presence of cases (trees with symptoms of *P. ramorum* canker) and controls (asymptomatic trees) in the study area, and absence of recent disturbances that might affect tree health (e.g., root-damaging construction). Plots were established in areas where *P. ramorum* had been shown to be prevalent. Coast live oak was the subject host species at 10 of the 12 locations; tanoak was the subject species at the remaining two locations.

Table 1. Locations of plots and host species studied.

Location number	Location	County	Approximate latitude and longitude	Number of plots	Subject tree species
1	Marin Municipal Water District (MMWD) watershed - Azalea Hill area	Marin	37.9723 N 122.6274 W	12	coast live oak
2	MMWD-Pumpkin Ridge south	Marin	37.9527 N 122.5949 W	16	coast live oak
3	MMWD-Pumpkin Ridge north	Marin	37.9599 N 122.5989 W	11	coast live oak
4	MMWD-Phoenix Lake area	Marin	37.9590 N 122.5770 W	11	coast live oak
5	China Camp SP - Miwok Meadows area	Marin	38.0044 N 122.4848 W	16	coast live oak
6	China Camp SP - SE Buckeye Point area	Marin	38.0044 N 122.4768W	12	coast live oak
7	Woodacre (Private land)	Marin	38.0175 N 122.6472 W	12	coast live oak
8	Lucas Valley (Private land)	Marin	38.0432 N 122.5996 W	12	coast live oak
9	Muir Woods NM / Mt. Tamalpias SP	Marin	37.9024 N 122.5839 W	10	tanoak
10	Wall Road (Private land)	Napa	38.4092 N 122.4751 W	13	coast live oak
11	Marin County Open Space land, Novato	Marin	38.0988 N 122.6273 W	13	coast live oak
12	Jack London SP	Sonoma	38.3450 N 122.5616 W	12	tanoak

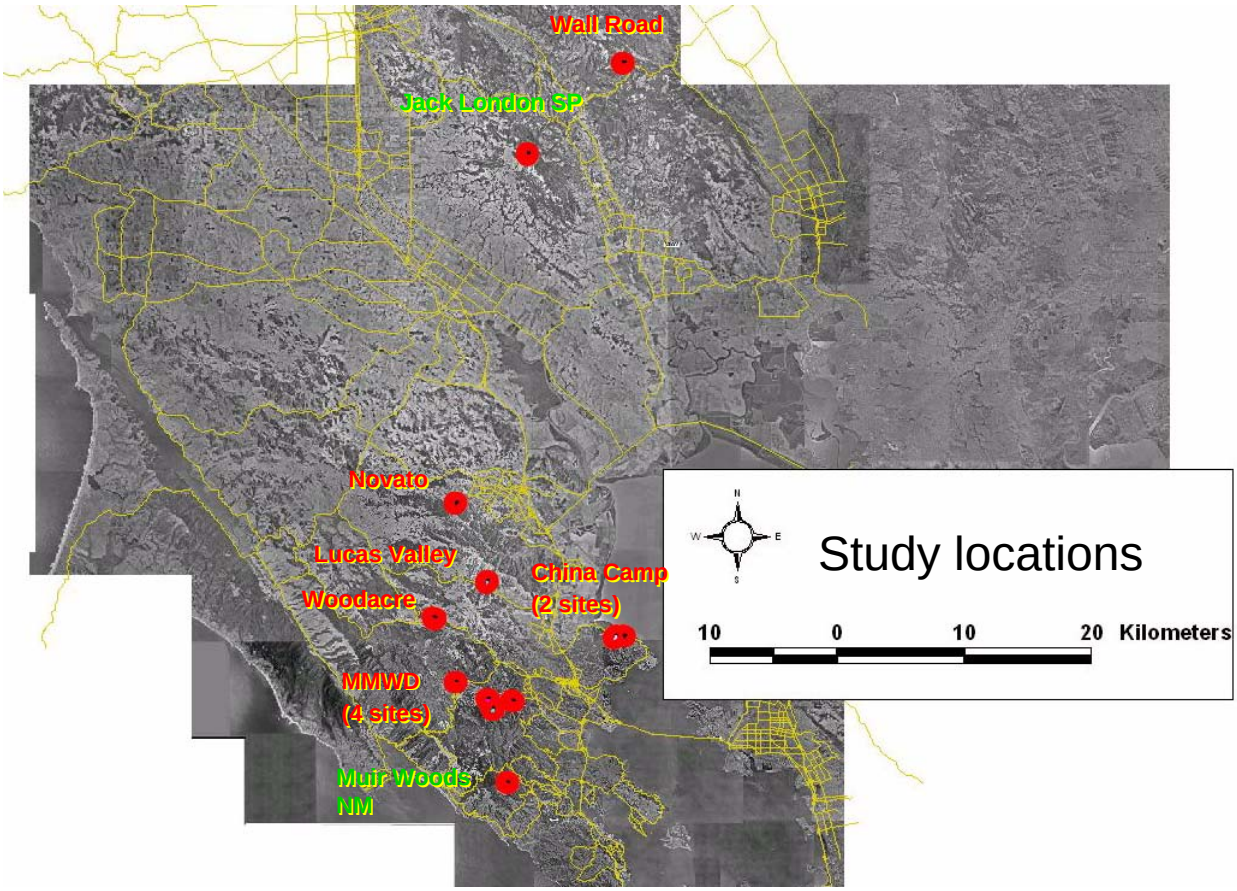

Figure 1. Map showing locations of study areas in Marin, Napa, and Sonoma counties. Background image is a mosaic of USGS digital aerial orthophotos.

Plot selection

At each study location, we established 10 to 16 circular 8 m radius (0.02 ha) fixed-area plots, each of which was centered at a subject tree. The number of plots per location was limited by the time constraints associated with making stem water potential measurements. After determining that symptomatic trees (cases) were present in adequate numbers in the stand, we established a random starting point and searched for the nearest case or control tree starting from that point. This tree became the first subject tree and the center of the first plot. Subsequent tree-centered plots were spaced approximately 25 m apart. Actual interplot spacing varied with vegetation and terrain, but to avoid overlap between plots no two adjacent plots were located closer than 16 m apart. We attempted to alternate case and control plots, but if the designated subject tree type (e.g., control) did not exist within a 4-8 m search radius of the target point, the other subject type was selected. Potential cases and controls were rejected if they did not have foliage low enough to be accessed for water potential measurements.

The distribution of plots across the landscape varied by location. In general, we attempted to distribute the plots across a range of topographic positions, slopes, and aspects. We marked the subject tree in each plot with a numbered aluminum tree tag. Tags were placed at varying heights, but generally point toward the next successive plot. To help relocate subject trees within each study site, we recorded the distance and azimuth readings between plots. We subsequently determined the coordinates of the plots at each location using a GPS receiver with an external, mast-mounted antenna, although the position of some plots at location 9 could not be determined with GPS due to poor satellite reception.

Stem water potential measurements

In September of each year (2000-2003), we collected midday stem water potential (SWP) readings on the center subject tree in each plot during the peak midday period (about 1300-1530 PDT) following methods outlined by Shackel (2000). In addition, starting in 2001, readings were made on 45 additional trees located within plots for comparative purposes. On each tree, we selected a minimum of two shoot tips with several leaves for measurement. We selected shoots and leaves that branched directly off the trunk, from main branches near the trunk, or from basal sprouts (primarily for tanoak). Each shoot tip was sealed in a clear plastic bag and overbagged with a larger opaque reflective plastic bag. These bags prevent the leaves from transpiring and overheating. Bags were left in place for 2 or more hours to allow leaf water potential to equilibrate to that of the subtending stem. At the time of the reading, the outer opaque bag was removed and the shoot tip was excised and placed into the pressure chamber while still sealed in the inner plastic bag. Two SWP readings were made on most trees. In general, two valid SWP measurements from a single tree were within 0.05 to 0.1 MPa of each other. SWP measurements were made with a pump-up pressure chamber (PMS Instrument Co., Corvallis OR) fitted with a 10.2 cm diameter 40 bar (0.4 MPa) gauge with 1% accuracy full scale.

Stem water potential readings can vary from day to day due to differences in daily vapor pressure deficits (VPD). To estimate VPD during the period that SWP readings were made, we recorded minimum and maximum temperature and relative humidity values during this period using a portable electronic thermohygrometer (Mannix TH Pen, model PTH8708). The relative humidity readings of the thermohygrometers were calibrated using a sling psychrometer. In all years, one thermohygrometer was placed in a vented shelter mounted on a mast and was positioned near the upper portion of the tree canopy layer during the observation period. Starting in 2001, we used a second shaded thermohygrometer mounted about 1.5 - 2 m above the ground to measure conditions below the canopy and determine whether VPD varied with position in the canopy during the measurement period. VPD was calculated from the average of the recorded minimum and maximum temperature values using the following formula:

$$VPD\ (KPa) = [0.6108 \times e^{(17.27T/(T+237.3))}] \times (1-RH/100) \qquad \text{(Equation 1)}$$

where:
T = average temperature (degrees Celsius)
RH = average relative humidity.

Bark characteristics

In 2003, we collected data on physical characteristics of the bark of coast live oak trees at all locations except 9 and 12, which did not include coast live oaks. Bark morphology data were collected for the subject tree in each plot and on additional plot trees used for water potential measurements. Bark characteristics recorded are presented in Table 2 below.

Table 2. Bark morphological characteristics rated for coast live oak trees in 2003.

Variable	Trees rated[1]	Year(s) evaluated[2]	Method	Scale/units and notes
Lichen abundance (lower 2m of bole)	S,A	2003	visual ranking of lichen cover	(0) none; (0.5) trace; (1) low; (2) moderate to high
Moss abundance (lower 2m of bole)	S,A	2003	visual estimate of moss cover	(0) none; (0.5) trace; (1) low; (2) moderate to high
Moss location	S,A	2003	visual assessment	(1) basal only (lower 1-2 m of bole) (2) extending up bole into upper bole and/or canopy
Type of bark fissures present	S,A	2003	visual assessment	(1)shallow; (2) medium; (3) deep
Brown bark from recent bark expansion in fissures	S,A	2003	visual assessment	present/absent
Deep bark crack	S,A	2003	visual assessment	present/absent (Unlike fissures, cracks are abrupt discontinuities that extend deep into the bark or to the cambium that are not associated with normal growth patterns.)
Bark texture	S,A	2003	visual description	bark texture was described using one or more of the following characteristics: smooth, irregular, striate, checkered, corky, furrowed

[1]Tree types: S=subject tree; A=additional trees used for water potential readings starting in 2001.

Bark thickness was measured using a needle-type bark probe (Figure 2), which functioned in the same way as the one described by Gill and others (1982). 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 which had been used for increment growth measurements in 2002. 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. This data was analyzed to determine the optimal height for measuring bark thickness.

Based on the results from the pilot study (discussed in Results below), we selected 1m 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 in the extra trees used for water potential measurements. Including the data for the 19 trees in the pilot study, bark thickness data was gathered on a total of 81 trees in 2003. Bark thickness of live trees was measured with the bark probe. For dead trees, gaps due to separation between wood and overlying bark due to drying can lead to erroneous readings using the probe, so bark was chipped open so that its thickness could be measured while viewing the bark in a radial or cross section. The status of the bark at each point of measurement (live, dead but moist, dead and dry) was recorded.

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 fresh sections of coast live oak stems that were then allowed to air dry. After drying, bark was cut away at the point where the bark probe measurement had been made and bark thickness was remeasured. The moisture status of the bark was visually estimated as done for field samples (moist or dry). The percent shrinkage in thickness was used to adjust dead bark thickness measurements for moist or dry bark samples to estimated fresh bark thickness.

Figure 2. Bark probe used to measure bark thickness, adapted from the design used by Gill and others (1982). The square brass tip of the probe is placed against the surface of the bark and a blunt-tipped 1.9 mm diameter steel probe (constructed from a bicycle tire spoke) is pressed through the bark. The probe tip stops when it reaches the wood and the depth of the probe is read to the nearest mm from gradations engraved in the outer brass barrel of the probe.

Additional tree and plot variables

Data collected on subject trees are listed in Table 3. The same information collected for subject trees was also collected for additional plot trees used for water potential measurements and cored control trees located beyond the plots. In 2003, we also assessed the vertical distribution of cankers along the bole of coast live oak study trees. Bleeding and external bark necrosis were primarily used to delimit the extent of canker margins. Chipping at canker margins was minimized to avoid affecting future observations on study trees. We recorded the apparent height above the soil level for the lower margin of the lowest *P. ramorum* canker and the upper margin of the highest *P. ramorum* canker on each tree.

The disease status of all coast live oak, black oak, and tanoak trees within plots was evaluated in September each year. In 2002, we recorded the distance and azimuth coordinates of each plot tree from the plot center to facilitate tracking of disease progress in these trees. Several additional plot variables were evaluated for each plot tree starting in 2002. Table 3 indicates which disease and tree variables were evaluated in plot trees.

Plot-related variables were assessed on an 8 m radius fixed-area plot centered at the subject tree. Table 4 lists the variables we evaluated for each plot. Most plot variables were initially evaluated in 2000, but some additional plot variables were added in subsequent years. We evaluated regeneration of coast live oak, black oak, and tanoak within the plots each year. Most other plot variables (e.g., density of non-host tree species) have not changed substantially since the beginning of the study. These variables were not reevaluated unless evidence of change was seen.

We used plot slope, aspect, elevation, and latitude data to calculate the total annual insolation (solar radiation) that the plot would receive in the absence of shading from vegetation or nearby landforms. Annual insolation quantitatively integrates the effects of plot slope and aspect. Daily insolation was calculated for each day of the year and all values were summed to calculate annual insolation. Insolation was calculated using a program developed by Dr. Tom Rumsey (Dept. of Biological and Agricultural Engineering, UC Davis) based on the Hottel estimation model (Duffie and Beckman 1991). We reprogrammed Dr. Rumsey's original Fortran program into Paradox® ObjectPAL. Other derived variables are described in the results.

Statistical analyses

We used JMP® 5.0.1.2 statistical software (SAS® Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $P \leq 0.05$.

We used logistic regression models to identify predictors of disease and disease progress outcome variables. Development of these models was described in our previous report (Swiecki and Bernhardt 2002a). We tested modifications of the previously reported models by substituting related variables in the models with new variables being tested. We calculated Akaike's information criterion (AIC) to compare the fit of alternative models using different sets of variables. For models constructed for a given data set, smaller AIC values indicate better model fit.

We used the likelihood ratio chi square test to test for independence of variables in 2 × 2 or larger contingency tables. We used linear regression and analysis of variance models to test for associations between continuous outcomes (e.g., SWP) and continuous or categorical predictor variables. We also used analysis of variance (F-tests) or t-tests to test whether mean levels of continuous variables differed between cases and controls.

Table 3. Tree variables measured for subject trees, other plot trees, and out of plot trees used for increment coring.

Variable	Trees rated[1]	Year(s) evaluated[2]	Method	Scale/units and notes
General tree descriptors				
Tree species	S,A,P: C:	2000 2002		*Q. agrifolia, L. densiflorus or Q. kelloggii* (plot trees only)
Origin class	S,A: C:	2000 2002	visual assessment	seed (0) or sprout (1)
Distance to plot center	A: P,C:	2001 2002	laser rangefinder	m; recorded for plot trees in 2002
Azimuth to plot center	A: P,C:	2001 2002	compass	degrees; recorded for plot trees in 2002
DBH	S: A: P,C:	2000 2001 2002	flat tape measure	cm
Sky-exposed canopy	S: A: P,C:	2000 2001 2002	visual estimate	pretransformed 0-6 scale[3]; percent of canopy projection area with unobstructed access to direct overhead sunlight
Number of stems from ground	S: A: P,C:	2000 2001 2002	count	stems/tree

Table 3. Tree variables measured for subject trees, other plot trees, and out of plot trees used for increment coring (continued).

Variable	Trees rated[1]	Year(s) evaluated[2]	Method	Scale/units and notes
P. ramorum canker-related symptoms				
Phytophthora-related symptoms	S,A,P: C:	2000-on 2002-on	visually assess symptoms present	(0) No symptoms (1) Early - bleeding cankers only (2) Late - cankers plus beetle boring and/or *H. thouarsianum* (3) Dead as result of *Phytophthora* infection; evidence of bark cankers present
Recent bleeding from cankers	S: A: P:	2000-on 2001-on 2002-on	visual assessment of exudate	Present (1) scored if bleeding appeared to have occurred within the previous 4-6 months / otherwise absent (0)
Phytophthora canker count	S: A:	2000-on 2001-on	count	Estimated on basis of external bleeding spots and limited inspection of canker margins. In 2000, only an overall count for all stems was made. In 2001, counts per stem for multistemmed trees were also made.
Percent girdling due to *Phytophthora* cankers	S,A:	2000-on	visual estimate	pretransformed 0-6 scale[2] Percent of circumference affected estimated based on projection of cankered areas as if all were viewed on same cross section; some limited chipping of bark done to confirm horizontal extent of canker margins in some trees. In general, girdling ratings are difficult and less reliable on completely dead trees. In 2000, a single overall rating was made for all stems. Starting in 2001, individual ratings were also made for each stem of multistemmed trees.
Height of upper and lower *P. ramorum* canker margins above grade	S,A	2003	tape measure	Height (cm) above soil level was noted for the upper edge of the highest canker and lower edge of the lowest canker on symptomatic trees.
Stems with Phytophthora symptoms	S,A,P,C:	2000-on	count	infected stems/tree
Dead stems	S,A,C:	2000-on	count	dead main stems/tree and likely cause of stem death (Phytophthora canker or other)
Tree dead / cause	S,A,P,C:	2000-on	visual assessment	Causes: (0) not dead (1) Phytophthora canker; (2) other agent(s); (3) unable to determine (4) Phytophthora canker plus other agent(s) Tree scored as dead if all main stems are dead, even if small live basal sprouts are present.
Hypoxylon thouarsianum Percent girdling	S: A: P,C:	2000-on 2001-on 2002-on	Visual estimate based on presence of fruiting bodies	pretransformed 0-6 scale2 Percent of circumference affected estimated based on projection of cankered areas as if all were viewed on same cross section;
Hypoxylon thouarsianum Greatest density in 0.1 x 1 m vertical strip	S,A,C:	2002	count	Count of fruiting bodies. Individual lobes counted separately.

Table 3. Tree variables measured for subject trees, other plot trees, and out of plot trees used for increment coring (continued)

Variable	Trees rated[1]	Year(s) evaluated[2]	Method	Scale/units and notes
P. ramorum canker-related symptoms (continued)				
Wood boring beetles in main stem	S,A,P,C:	2000-on	Shape and size of exit holes	Type of beetle based on shape of exit holes
Abundance of bark and/or ambrosia beetles in main stem	S: A: P,C:	2000-on 2001-on 2002-on	presence of boring dust and/or holes	(0) none seen (1) low (2) moderate (3) high
Other tree condition variables				
Canopy thinning	S A C	2000-on 2001-on 2002	visual estimate	0-2 Scale: (0) none; (1) slight; (2) pronounced
Canopy dieback	S A P C	2000-on 2001-on 2002-on 2002	visual estimate	pretransformed 0-6 scale[3] Based on percent dead crown volume
Severe tree decline due to other agents	S,A,P C	2000-on 2002	visual assessment	yes (1)/ no (0) Trees scored in decline if overall condition is poor enough that death within 10 years was judged to be likely.
Decay impact	S A C	2000-on 2001-on 2002	visual assessment	0-3 Scale: (0) none; (1) low; (2) moderate: (3) high Decay impact rating (Swiecki and Bernhardt 2001a) assesses the probability that existing decay will have a significant negative impact on tree health or survival. Assessment of decay impact is 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. Levels were recoded to three classes as follows for some analyses: (1) none; (2) low or moderate; (3) high
Status change	S,A,P	2000-on	comparison of 2000 and 2001 data	Evaluation based primarily on canker extent, colonization by secondary organisms, and dieback. (0) no change; (1) improved condition; (-1) degraded condition
Epicormics	S: A: C:	2000-on 2001-on 2002	visual assessment	0-2 Scale: (0) none; (1) few; (2) numerous
Live basal sprouts	S,A,P:	2000-on	visual observation	presence (1) / absence (0) scored for dead trees only Trees are scored as dead if all main stems are dead even if some live basal sprouts are present.
Other agents and symptoms	S,A,P,C:	2000-on	visual observation	Presence of wood decay fungi fruiting bodies and canker rot or root rot symptoms were noted.

Table 3. Tree variables measured for subject trees, other plot trees, and out of plot trees used for increment coring (continued)

Variable	Trees rated[1]	Year(s) evaluated[2]	Method	Scale/units and notes
Other tree condition variables continued				
Defect codes	S,A: P: (if failed)	2002-on	visual observation	The presence of various structural defects that may contribute to the risk of tree failure were coded. (1) Dead branch or branch stubs (2) Multiple trunks/ codominant stems (3) Hollow branch stubs (4) Dense crown (5) Heavy lateral limbs/ excessive branch end weight (6) Uneven branch distribution: one sided (7) Uneven branch distribution: top heavy (8) Multiple branches from same point (9) Embedded bark in crotch (10) Crook or sweep (11) Leaning trunk (12) Cracks or splits (13) Kinked or girdling roots (14) Cavity (15) Decay column
Tree failure	S,A,P:	2000-on		Failures of bole or branches >20 cm diam noted if present
Failure type	S,A,P:	2001-on		(1) Root (2) Root crown (lower edge of fracture is near soil surface) (3) Bole (main stem) (4) Scaffold (lowest first order branches arising from bole) (5) Branch (all other branches)
Tree condition at time of failure	S,A,P:	2001-on	based on condition of twigs and foliage	(1) Live (2) Dead (3) Uncertain
Estimated failure date	S,A,P:	2001-on	based on weathering of failed surface, degradation of failed part, previous observations, etc.	(1) within previous 6 months (2) 6-12 months prior to rating More precise dates were estimated if supportable by observations (e.g., green foliage on failed part)

[1]Tree types: S=subject tree; A=additional trees used for water potential readings starting in 2001; P=other plot trees; C= trees located beyond plot edges used for coring in 2002 (Swiecki and Bernhardt 2003b) and bark probe measurements in 2003. Only asymptomatic trees were chosen for coring in 2002.

[2]Variables scored in a single year were reevaluated only for trees which showed a change from the original values.

[3]The 0-6 scale is based on the following arcsine-transformed percentage scale:

(0) Symptom not seen
(1) < 2.5%
(2) 2.5% to <20%
(3) 20% to < 50%
(4) 50% to < 80%
(5) 80% to < 97.5%
(6) 97.5% to 100%

Table 4. Plot and stand variables measured in study plots. Except as noted, all variables were measured in the 8 m radius fixed-area plots.

Variable	Year(s) evaluated[1]	Method	Scale/units and notes
Tree density / species composition	2000	count by species	Trees have at least one stem at least 3 cm DBH located within 8 m of plot center; multi-stemmed trees count as single trees; coppiced redwoods separated by at least 1 m count as separate trees
Plot slope	2000	clinometer	percent
Plot aspect	2000	compass	degrees
Plot drainage	2000	visual observation	none; creek/drainage with surface water; dry creek or drainage
Plot drainage proximity	2000	visual observation	0 if in plot; otherwise estimate meters from plot edge
Plot tree canopy cover	2000	visual estimate	pretransformed 0-6 scale[2]; overall tree cover in plot
California bay cover	2002	visual estimate	pretransformed 0-6 scale[2]; bay cover in plot, including regeneration
Madrone cover	2002	visual estimate	pretransformed 0-6 scale[2]; madrone cover in plot, including regeneration
Woody understory cover	2000	visual estimate	pretransformed 0-6 scale[2]; includes both shrubs and small (<3 cm DBH) tree regeneration
Plot shrub cover	2001	visual estimate	pretransformed 0-6 scale[2]
Poison oak cover	2002	visual estimate	pretransformed 0-6 scale[2]
Overstory canopy trees species in plot	2001	visual assessment	list of species Overstory canopy trees do not have to be rooted within the plot.
Count by general tree health class (trees other than SOD hosts[3])	2000, 2001	tree count by species, subcategorized by symptom class and canopy position (overstory/understory)	Symptom classes: (1) live (2) decline (3) dead
SOD host[3] regeneration	2000-on	count or estimate if >10	regeneration = seedlings and saplings <3 cm dbh
Disease incidence in SOD host[3] regeneration	2000-on	count or estimate percent if count > 10	Disease may be due to *P. ramorum* and/or other agents or factors
Dead SOD host[3] regeneration	2000-on	count	Cause of mortality in regeneration was not determined
Regeneration of trees other than SOD hosts[3]	2000	presence noted by species	regeneration: seedlings and saplings <3 cm dbh
Other pathogens/agents	2000-on	note presence	listing of agents and symptoms observed, including various decay fungi, canker rot, root disease, *H. thouarsianum*, and beetles
Woody understory species	2001	note presence	list shrubs and woody vines present within plot; herbaceous species and grasses were not scored
Disturbance	2000	Note type of disturbance	roads, trails, logging, etc. within plot or near edge of plot were noted
Oak/tanoak failure in plot	2001	count	Bole and large limb failures (>20 cm diam) observed in the plot were noted.
Basal area[4]	2000	survey laser reticle	reticle BAF = 5 m^2/ha

[1]Variables scored in a single year were reevaluated only for trees which showed a change from the original values.

[2]The 0-6 scale is based on the following arcsine-transformed percentage scale:

0: Symptom not seen
1:< 2.5%
2: 2.5% to <20%
3: 20% to < 50%
4: 50% to < 80%
5: 80% to < 97.5%
6: 97.5% to 100%

[3]SOD hosts = hosts of *P. ramorum* stem canker, i.e., coast live oak, black oak, and tanoak

[4]Basal area measurements were made on a variable-radius plot centered at the subject tree.

RESULTS AND DISCUSSION

Stem water potentials (SWP) of coast live oaks and tanoaks

Comparison of average stem water potentials 2000 - 2003

On average, coast live oak and tanoak showed lower levels of water stress (i.e., less negative stem water potentials [SWP]) in September 2003 than in September 2001 and September 2002. However, the lowest water stress levels observed to date were in the initial year of the study, September 2000 (Figure 3). At each location we gathered climate and relative humidity data during the 2 to 2.5 hours during which we measured SWP. Due to warmer temperatures and lower relative humidities, vapor pressure deficits measured each September have increased each year of the study (Figure 4). For example, average September temperatures during the data collection period in September 2003 was on average 5.6°C warmer than in September 2000 (Figure 5). The two tanoak locations in the study (Table 1) are normally cooler and more humid than the coast live oak locations. However, during the September 2003 SWP measurements, both of the locations had unusually high temperatures and low humidities, resulting in high vapor pressure deficits (Figures 4, 5).

Although SWP can be influenced by vapor pressure deficit at the time of measurement (McCutchan and Shackel, 1992), our data have consistently shown little or no influence of vapor pressure deficit on SWP. Furthermore, seasonal ETo levels are also not well correlated with average September SWP measurements (Figure 6). Comparing Figures 3 and 6, it appears that rainfall is the main driving force behind SWP measured in September. Rainfall totals from the previous season are generally correlated with average September SWP levels, but residual effects from previous years' rainfall also appear to have an influence on SWP. A three-year running average that weights the previous two seasons' precipitation at 2/3 and 1/3 of the current season's rainfall (Figure 6) shows the same overall trend seen in the SWP data. These weighted averages may more accurately reflect the depletion and recharge of soil moisture deeper in the soil profile. This moisture deep in the profile probably has a strong influence on tree water stress at the end of the season because most or all of the more shallow available water has been depleted by that time.

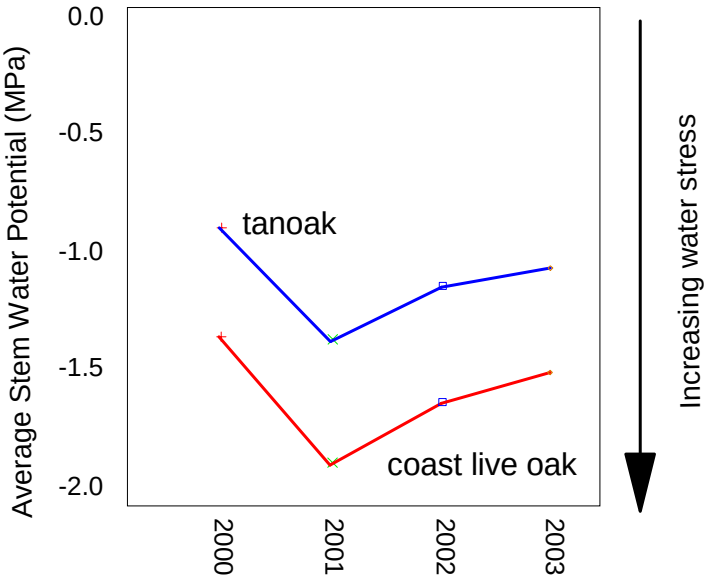

Figure 3. Average stem water potential for coast live oak and tanoak subject trees across all locations and years.

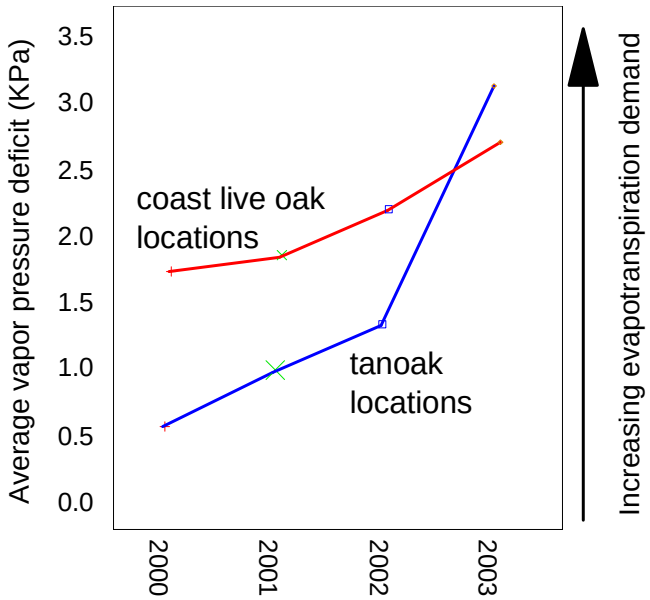

Figure 4. Average vapor pressure deficit (KPa) calculated from temperature and relative humidity measurements recorded in September of each year during stem water potential measurements at tanoak and coast live oak study locations.

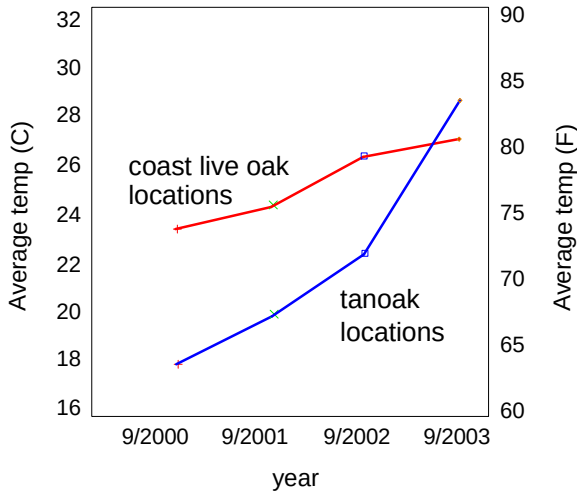

Figure 5. Average temperature recorded in September of each year during stem water potential measurements at tanoak and coast live oak study locations.

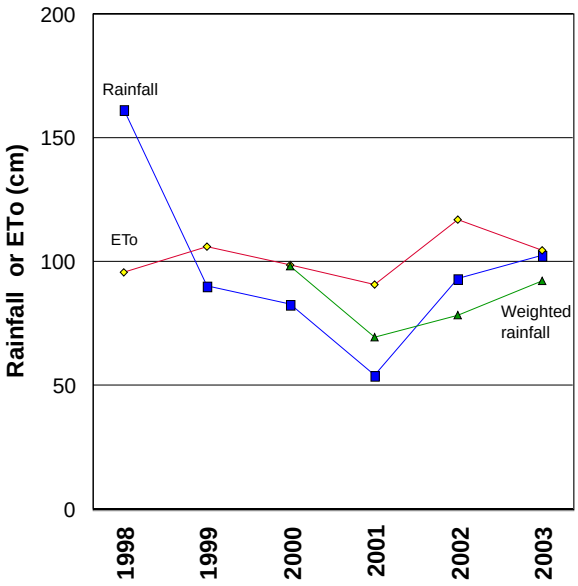

Figure 6. Rainfall and potential evapotranspiration (ETo) data for all study locations. Blue line represents the average rainfall (September of previous year through August of listed year) for all locations, calculated from the nearest available weather station data for each location. Weighted rainfall is a weighted three year running average of seasonal rainfall. Weights are 3x for current season, 2x for previous season and 1x for the season before that. ETo data is from the California Irrigation Management Information System (CIMIS) station in Santa Rosa.

As seen in other years, SWP readings of individual trees were highly correlated between 2002 and 2003 (adjusted $R^2 = 0.694$, $F<0.0001$). However, the correlation between multiple trees within plots was lower than observed in some previous years. Nonetheless, the correlation of tree SWP levels within plots was still significant (adjusted $R^2 = 0.507$, $F<0.0001$). Predictors of SWP have been discussed in previous reports (Swiecki and Bernhardt 2001a, 2002a, 2002b, 2003b), and include annual insolation, Phytophthora girdling rank, decay impact rating, and sky exposed canopy rating.

Disease status and SWP

Average SWP of trees in the disease classes show in Figure 7 have generally maintained the same relative rank over the past four years. This supports one of our original assumptions for this study: SWP potentials measured on trees with only early *P. ramorum* canker symptoms should be representative of pre-infection SWP levels because *P. ramorum* canker is not likely to affect SWP in the early stages of disease. We have also directly observed SWP levels before and after *P. ramorum* canker development in two study trees. Both of these showed similarly high SWP readings before and after *P. ramorum* canker symptoms developed.

The relationship between SWP and disease status seen in 2003 continued the trend seen in previous years. Trees with symptoms of *P. ramorum* canker continue to have higher average SWP levels (i.e., lower water stress) than asymptomatic trees (Figure 7). As noted previously (Swiecki and Bernhardt 2001a, 2002a, 2002b, 2003b), this suggests that water stress is not a significant predisposing factor for the development of *P. ramorum* canker in coast live oak. Hence, these results show that low soil moisture conditions do not increase *P. ramorum* disease risk, at least within the study areas. In fact, the data and our associated observations indicate that the reverse situation commonly occurs: trees in sites that are relatively dry due to soil and topographic factors typically have a low risk of developing *P. ramorum* canker. Furthermore, because most trees that developed *P. ramorum* canker had both relatively high

SWP (low water stress) and high canopy exposure (high potential transpiration rates) (Swiecki and Bernhardt 2001a, 2002a, 2002b), it is unlikely that these trees had any significant impairment of root function prior to infection. This suggests that severe root disease is not a predisposing factor for *P. ramorum* canker in the study areas.

Figure 7 shows only trees that had the same disease status in all four years of the study. However, trees that showed progress in *P. ramorum* canker symptoms over the past four years (e.g., progression from asymptomatic to early or from early to late) did not show any consistent deviation from the overall pattern of SWP variation over time seen in Figure 7. The only exception to this pattern was seen in two trees that were in the final stage of decline that occurs right before total canopy death at the time SWP measurements were made. Both of these trees had only a few remaining green leaves and SWP readings showed greatly increased levels of water stress compared to their readings in previous years.

P. ramorum cankers are primarily in the bark, but they can affect the outermost layer of xylem tissue (Rizzo and others 2002b). Most xylem destruction in *P. ramorum*-infected trees occurs through the action of wood decay fungi, most commonly the sapwood-decaying fungus *Hypoxylon thouarsianum*. Trees in late stages of disease (i.e., trees with beetles and/or *H. thouarsianum* sporulation) appear to compensate for loss of water-conducting tissue by shedding leaves, which may partially account for the sparse canopies seen among trees which persist in late stages of disease for several years. Leaf shedding allows trees to maintain relatively high SWP levels by reducing total leaf area and hence transpiration. However, when the decay of a diseased tree's xylem passes a critical threshold, water transport to the top is cut off and the tree quickly dries out.

The SWP data suggest that in most coast live oak affected by *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. If loss of root function was the cause of canopy dieback, we would expect SWP levels to be lower as disease progressed. In the final stages of tree decline, decline and death of the root system is likely to occur. Nonetheless, many coast live oaks and tanoaks whose tops have been killed by *P. ramorum* canker or which have had bole failures related to *P. ramorum* canker have produced basal sprouts that have survived for several years. This indicates that the root systems of these trees have not yet died. These data are consistent with observations by others (D. Rizzo, personal communication) that *P. ramorum* has not been shown to cause root decay in naturally-infected woodlands, even where the disease is common.

Trees declining due to factors other than *P. ramorum* consistently had the highest SWP readings, i.e., lowest levels of water stress (Figure 7). Many of these severely declining trees (rated as likely to die within the next 10 years) are suppressed understory trees, often with reduced canopies. It appears that high SWP in these trees is due to low transpiration rates associated with heavy canopy shading and low total leaf area.

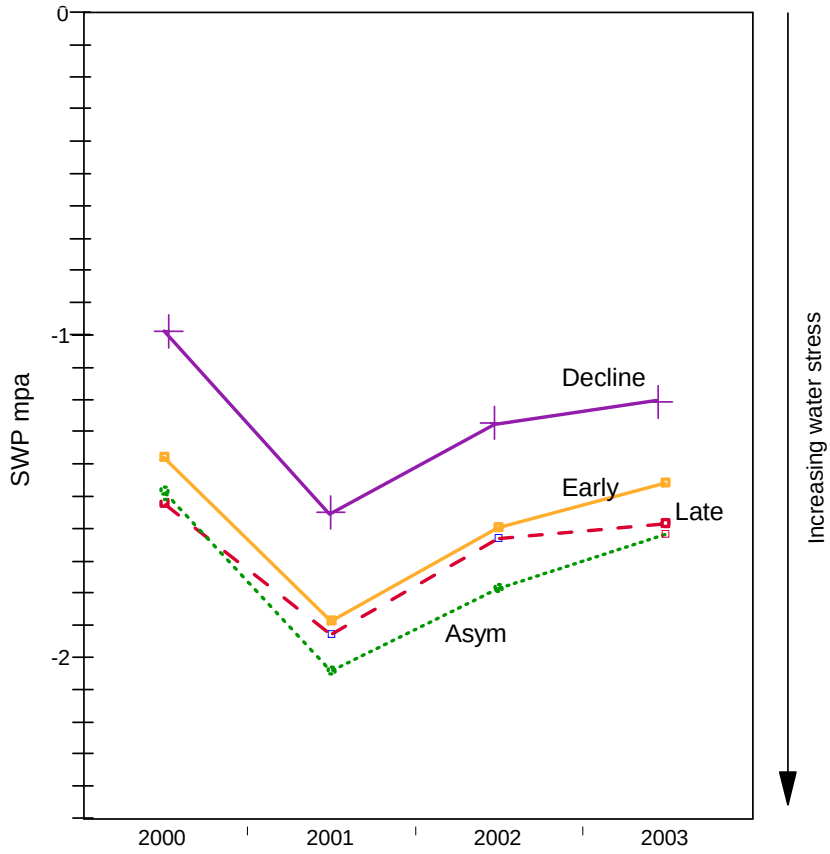

Figure 7. Stem water potential of coast live oak in September of each year for coast live oak trees that had the same disease status in all 4 years. Early = showing only bleeding cankers typical of *P. ramorum* infection; Late= bleeding cankers plus *Hypoxylon thouarsianum* or ambrosia beetles; Decline= no symptoms of *P. ramorum* infection but in severe decline due to wood decay fungi; Asym = trees lacking *P. ramorum* symptoms and not in severe decline.

Relationships between California bay and *P. ramorum* canker

Our previous reports (Swiecki and Bernhardt 2001a, 2002a, 2002b, 2003b) have documented that California bay cover and the count of bay trees in the plot are highly significant predictors of *P. ramorum* canker in coast live oak. A practical question arising out of this finding is, whether we can define a safe distance between a California bay tree and a coast live oak or black oak, i.e., a distance at which infection of the oak would be unlikely to occur. Determining a safe distance would be of immediate use to landowners that are attempting to protect individual trees and may have value in managing woodland stands to reduce disease risk.

We examined our existing California bay variables more closely to look at the feasibility of answering this question from our existing data. Our current variables include the number of live overstory bay trees and live understory bay trees in each plot; an estimate of bay canopy cover using a 0-6 scale; and binary variables that note whether bay is present in the plot and if bay regeneration is present in the plot.

All of these bay variables are highly correlated with each other. The count of overstory bay trees in the plot is the strongest predictor of the case outcome, i.e., disease (likelihood ratio p=0.0023, AIC =169). As we have previously reported, the risk of infection increases as the number of bay trees in the plot increases. Adding the count of understory bay trees to the count of overstory bay trees does not change

the variable enough to improve the predictive ability or fit of the model (likelihood ratio p=0.0022, AIC=169). In these plots, the low number of understory bay trees present does not appear to contribute substantially to additional infection risk. The presence of bay regeneration in the plot is not by itself a significant predictor of disease.

Our field observations suggest that in some cases understory bay that are directly adjacent to or overhanging oak stems may provide sufficient inoculum to initiate *P. ramorum* stem cankers in the oaks. Hence spatial distribution of understory bay within the 8 m radius plot may be important, although this is not accounted for in our current data.

The amount of bay cover in the plot (including both overstory and understory bay) is also a significant predictor of disease (likelihood ratio p=0.0120, AIC=172). Regression tree analysis of this variable shows that disease incidence increases in a stepwise rather than a linear fashion as bay cover increases. A binary variable for plot bay cover >20% is as good a predictor of disease (likelihood ratio p=0.0043, AIC=170) as the 0 to 6 bay cover rating. This suggests that 20% bay cover within an 8 m radius of a tree is a threshold that corresponds to about a 4-fold increase in disease risk, based on the odds ratio for multivariate models that include this variable. However, because disease was seen in trees that had no bay cover within the plot, infected bay foliage beyond 8 m from a subject tree appears to pose a risk of infection.

Although our current data show that risk of infection in coast live oak increases with the number of bay trees or bay cover present within 8 meters of the tree, it does not indicate what a "safe" distance might be. Given data by J. Davidson and D. Rizzo (personal communication) that shows a strong reduction in *P. ramorum* propagule density occurring between 0 and 5 m from bay canopy, it seems likely that model fit might be improved by accounting for the distribution of both understory and overstory bay canopy within and near the plot.

Vertical distribution of stem cankers

To help target the optimum height for studying the relationship between bark thickness and disease incidence, we collected data on the apparent height of the upper and lower margins of all *P. ramorum* cankers in subject trees (Figure 8). The lower margins of all of the cankers occurred within 1.3 m of the soil grade, with most (80%) occurring within the lower 0.4 m of the bole. The upper extent of the highest canker was quite variable, with an overall mean of 1.09 m above the soil grade. Only about 25% of the upper canker margins were above 1.5 m and none of the observed cankers extended beyond 2.2 m above soil level (Figure 8).

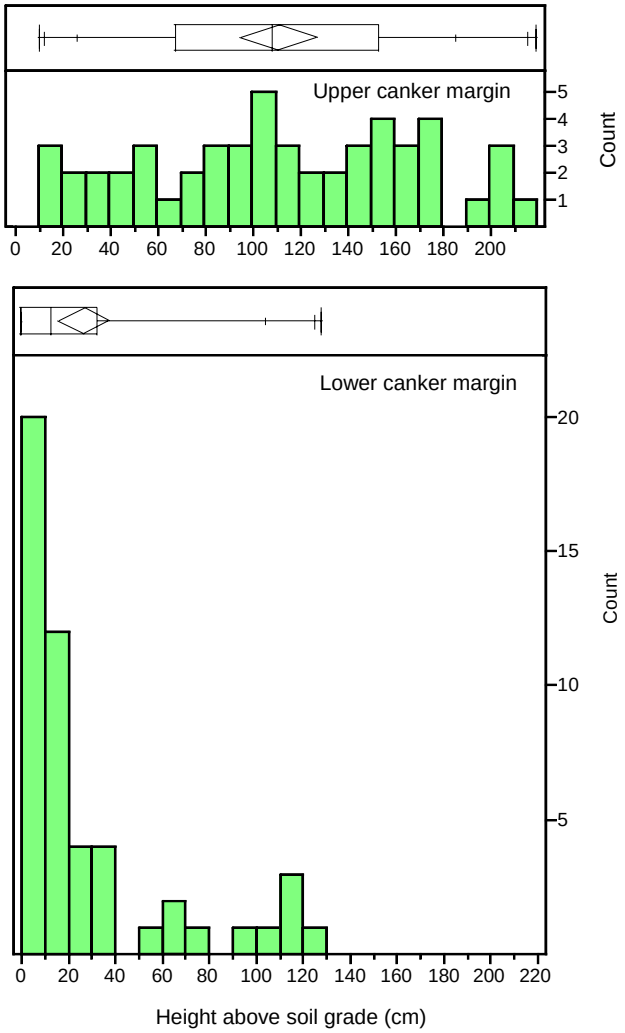

Figure 8. Frequency distribution of heights of upper (top graph) and lower (bottom graph) margins of stem cankers on study trees (n=52 for upper, 50 for lower). Box plots above each histogram indicate the median and 25th and 75th quantiles (line in center of box and box ends, respectively). The center of the diamond indicates the sample mean and the ends represent the 95% confidence interval of the mean.

Coast live oak bark characteristics and disease

Bark thickness characteristics

Initial bark thickness measurements were made at multiple heights on 19 healthy coast live oaks located beyond plot margins that had been cored in the 2002 pilot increment growth study (Swiecki and Bernhardt 2003b). We used analysis of variance to test for effects of height above ground and cardinal direction on bark thickness. 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 ($p<0.0001$). Although bark thickness decreased with increasing height, average bark thickness at 0.5 m (2.75 cm) was only 0.83 cm greater than the average bark thickness at 2 m.

Based on both the information on the vertical distribution of stem cankers and the bark thickness data from these initial measurements, 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, as shown above (Figure 8), most *P. ramorum* cankers on study trees are found near or below this height.

To adjust bark thickness readings of dead trees to account for shrinkage due to drying, we established an ongoing experiment to determine the degree of bark shrinkage in coast live oak bark. Preliminary average shrinkage coefficients from this study were 13% for samples rated as still somewhat moist (n=18) and 23% for samples rated as dry (n=11). Although these coefficients will be refined with additional measurements after all samples have dried completely, we used the existing coefficients as a first approximation to correct bark thickness measurements prior to the analyses discussed below.

Bark thickness and P. ramorum *canker*

Thirty-one of the 81 coast live oak trees whose bark thickness was measured had symptoms of *P. ramorum* canker. The mean adjusted bark thickness of these trees was significantly greater than that of the asymptomatic trees (t-test $p<0.0001$, Figure 9). Furthermore, if the degree of *P. ramorum* canker symptom development in 2003 is considered, mean bark thickness seems to be positively correlated with the rate at which disease progressed. Mean bark thickness was greatest among trees that had already died and lowest among trees that still had only early symptoms of disease in 2003. Trees with late symptoms in 2003 (cankers and secondary invasion by beetles and/or *H. thouarsianum*) showed intermediate bark thickness levels (Figure 9).

We will be collecting data on additional trees in 2004 to confirm this trend over a larger population, but the trend in the existing data suggests that coast live oak trees with thicker bark were more likely to become infected and that they developed severe disease symptoms more quickly than trees with thinner bark. It is notable that the same overall trends in bark thickness differences between asymptomatic and symptomatic trees are still apparent if bark thickness readings have not been corrected to account for bark shrinkage upon drying. For example, the difference between the mean bark thickness of symptomatic and asymptomatic trees is significant at $p=0.0181$ (t-test) for the raw (uncorrected) thickness readings.

Although an increase in adjusted bark thickness is associated with an increased risk of disease in coast live oak (likelihood ratio $p<0.0001$), bark thickness is also correlated with stem diameter (Figure 10). In coast live oak, bark thickness increases in a nonlinear fashion with increasing stem diameter (Figure 10), with an upper plateau near 5.5 cm. Relative bark thickness, i.e., the ratio of bark thickness to stem DBH, did not differ significantly between trees with and without symptoms of *P. ramorum* canker. Hence, in this sample, trees with *P. ramorum* canker generally had the same ratio of bark to wood as asymptomatic trees, but symptomatic trees were generally larger-diameter trees.

We have previously shown that stem cross-sectional area, which is related to stem diameter, is a predictor of disease risk in coast live oaks (Swiecki and Bernhardt 2002a, 2002b). In logistic regression models, bark thickness is a slightly better predictor of disease than stem diameter, but both are highly significant predictors in univariate models (likelihood ratio $p<0.0001$). In order to test bark thickness as a variable in multivariate disease risk models, we will need to collect data on bark thickness for the remaining subject trees. This is planned for the 2004 field survey season.

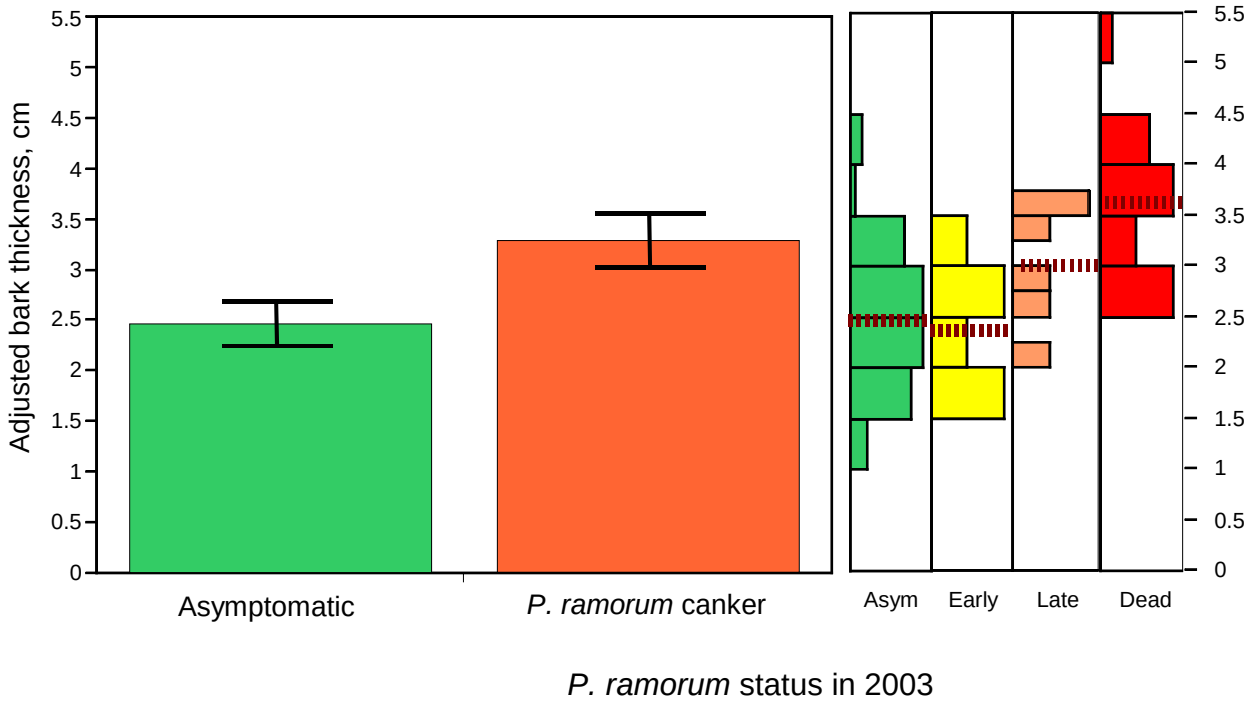

Figure 9. Relationship between adjusted bark thickness in coast live oak and *P. ramorum* disease status in 2003. Bark thickness readings were adjusted to account for shrinkage due to drying in dead bark samples. Bars at left show that average bark thickness at 1 m height in trees with *P. ramorum* canker symptoms in 2003 was significantly greater than that of asymptomatic trees. Error bars denote 95% confidence intervals of the means. Histograms at right show that average bark thickness was greatest in trees with the most advanced P. ramorum canker symptoms (late and dead). Dashed lines denote means of each group shown.

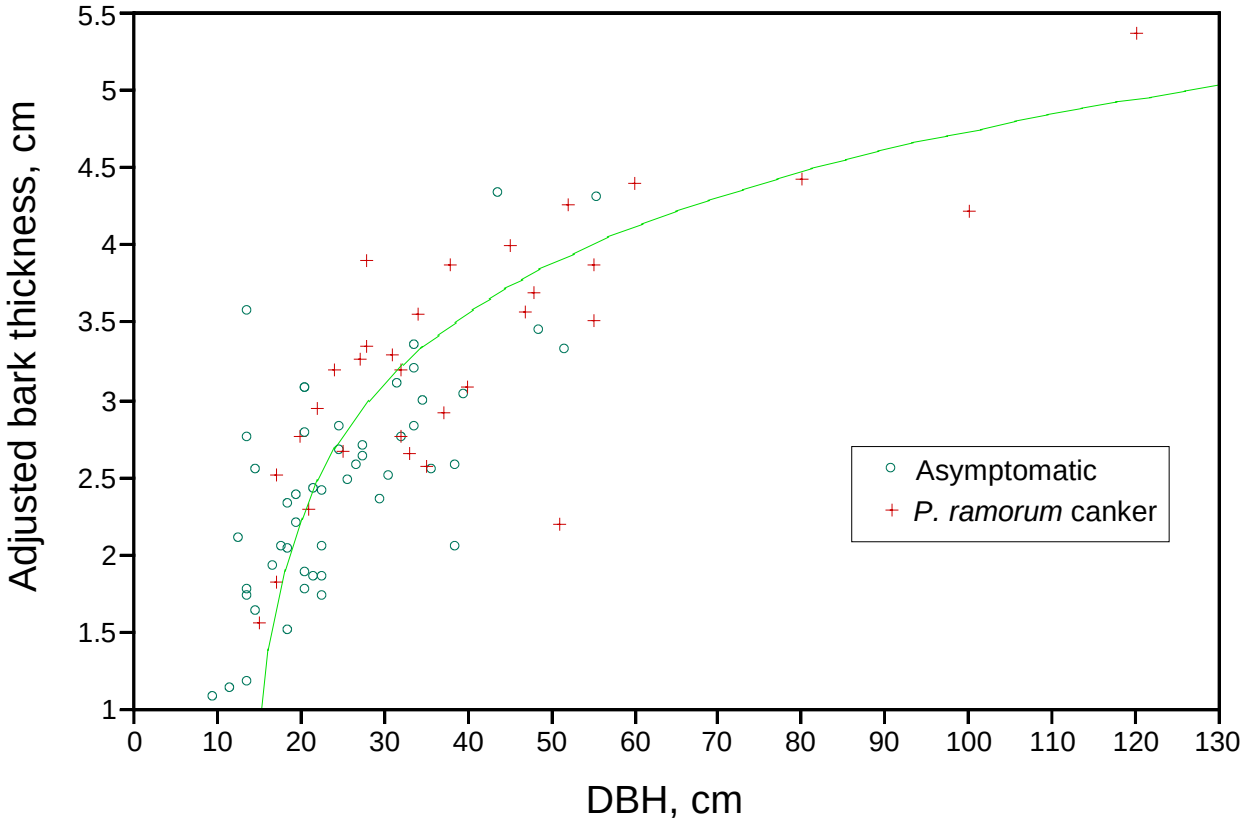

Figure 10. Relationship between bark thickness (at 1 m height) and stem diameter (at 1.37 m height, DBH) for coast live oaks with or without symptoms of *P. ramorum* canker. Fitted curve is $e^{(\text{adjusted bark thickness})} = -17.35691 + 1.3112428\ (\text{DBH})$; adjusted $R^2=0.69$, $p<0.0001$.

Bark morphology

In 2003, we attempted to classify the morphology of the bark of coast live oak subject trees using a variety of descriptors in order to determine whether surface bark characteristics could be related to disease risk. Almost all of these 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. Deep bark cracks, some of which extended to the wood, were not significant predictors of disease in logistic regression models, but have the potential to be sites of pathogen entry. Further observations on these cracks may therefore be warranted.

One bark characteristic was a significant predictor of the case outcome for 2000 through 2002. This was the presence of non-weathered, brown bark visible in the center of furrows or fissures caused by bark expansion (Figure 11). This characteristic appears to indicate that significant recent bark expansion has occurred. This factor was also a significant predictor in some multivariate models, notably models that included bay cover or density as a predictor of disease risk.

As we have reported previously (Swiecki and Bernhardt 2001b, 2002a, 2002b), several factors that are correlated with higher tree growth rates are also associated with increased disease risk. These include greater canopy dominance (higher sky exposed canopy), higher season-end water potential (low water stress), greater stem cross-sectional area, and the absence of severe decline due to other preexisting agents (e.g., severe canker rot). It seems likely that the bark expansion characteristic is similarly related to relatively high rates of radial stem growth.

We found that the bark expansion characteristic was positively correlated with sky exposed canopy. The mean sky-exposed canopy rating for trees with bark expansion (4.3) was significantly greater (t-test $p<0.0001$) than for trees without this characteristic (2.6), indicating that more dominant trees (high sky exposed canopy rating) were more likely to show the bark expansion characteristic. Furthermore, contingency table analysis showed that bark expansion occurred at significantly lower frequencies in trees that were rated as being in decline due to other agents in 2003 (likelihood ratio $p<0.0001$) or as having canker rot symptoms in 2003 (likelihood ratio $p=0.0107$). In multivariate logistic regression models including bay cover or density as a predictor, bark expansion was a better predictor of disease than canker rot presence or decline due to other agents, but was a poorer predictor than sky-exposed canopy ratings.

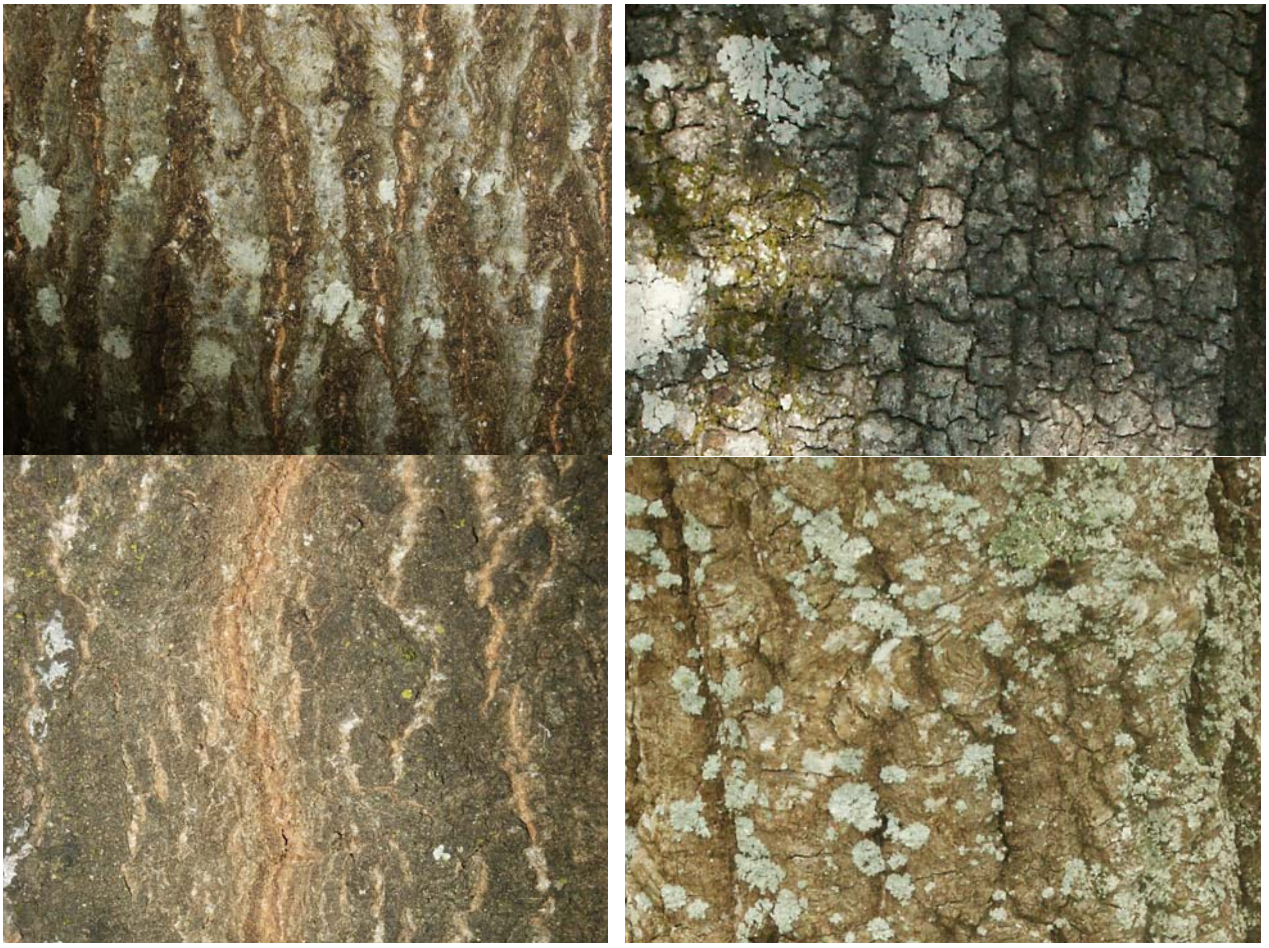

Figure 11. Coast live oak bark showing (left) or lacking (right) recent bark expansion based on the presence (left) or absence (right) of relatively unweathered brown bark within bark fissures or furrows. Tree bark at lower left shows especially wide fissure and generally wider brown areas than the bark at upper left. Overall bark morphology was described as striate (left photos), checkered (upper right), and furrowed (lower right).

Recent bark expansion is correlated with several variables suggesting that this character is an indicator of greater tree growth, which is correlated with increased disease risk (Swiecki and Bernhardt 2002a, 2002b). The bark expansion characteristic was qualitatively scored in 2003. Because this characteristic seems to have some potential for evaluating disease risk in coast live oak, in the 2004 field season, we will determine whether it can be quantified more precisely, and if so, whether the quantitative variable is a better predictor of disease risk than the qualitative presence/absence variable.

Disease progress

Coast live oak

For the period 1995-1998, seasonal precipitation at five local weather stations near our study plots averaged 141% of the 50 year average. Several of those high rainfall years (especially 1998) were characterized by frequent rains late in spring. These high rainfall years coincide with the beginning of the SOD epidemic. Over the first three years that our plots have been observed (2000-2002), average seasonal precipitation was 84% of the 50 year average. These conditions may not have been especially favorable for inoculum production or infection, and our plot data indicate that few or possibly no new infections have developed over this interval.

In the spring of 2003, the study areas experienced extended periods of wet weather, and most stations have reported rainfall accumulations near or above seasonal averages by the end of April. More importantly, spore trapping data from the UC Davis research group have shown that inoculum concentrations of *P. ramorum* detected in rainfall and in soil were far above levels detected in 2001 or 2002 (J. Davidson, personal communication).

Trees were reevaluated for symptoms of *P. ramorum* infection in September 2003. During the evaluations we isolated from 23 coast live oak trees. Of these, 11 were trees with possible new symptoms of *P. ramorum* infection and we obtained positive isolations for *P. ramorum* from 5 of these trees. This is the largest increase in newly-symptomatic trees since we began the study in 2000. A few of the symptomatic trees with negative isolations were provisionally scored positive for *P. ramorum* based on symptoms. Although it is possible the trees were infected prior to 2003, it seems more likely that most if not all of the newly-symptomatic trees were infected during the wet season in the early months of 2003.

We also isolated from 12 trees with bleeding canker symptoms which had not progressed or enlarged during the monitoring period (September 2000-2003). No positive isolations for *P. ramorum* were obtained from these trees. Ten of these trees have been scored as noninfected, and the remaining two trees were scored as positive for *P. ramorum* based on symptoms.

The graph in Figure 12 shows increases in the frequency of disease symptoms over time for all coast live oak study trees, based on the current diagnosis of each tree's infection status. Using September 2003 disease status data, it appears that the overall infection rate has increased from 22.9% in September 2000 to 24.4% in September 2003. The newly symptomatic trees observed in September 2003 account for about two-thirds of this relatively small increase in disease incidence.

Subject trees with late *P. ramorum* symptoms and trees declining due to factors other than *P. ramorum* in 2003 have developed progressively higher levels of canopy dieback over time (Figure 13). In contrast, trees with only early symptoms of disease in September 2003 did not show a significant increase in mean canopy dieback rating in 2003 compared to 2002 (matched pairs t-test). A number of trees with only early symptoms of disease have shown no recent canker expansion and in some cases, these cankers have apparently become inactive (no bleeding) and show strong callusing near the margins of the cankers. Some of these trees have shown increased vigor and recent shoot growth in the canopy, which has apparently led to a stabilization of dieback ratings. Levels of canopy dieback in asymptomatic trees have increased significantly between 2000 and 2003 (matched pairs t-test $p<0.0001$), showing the same overall magnitude of change as the trees with early *P. ramorum* symptoms (Figure 13). The causes of this increase in canopy dieback are not clear, but may be related to the change in soil moisture conditions from several years of above average rainfall to a period of below average rainfall.

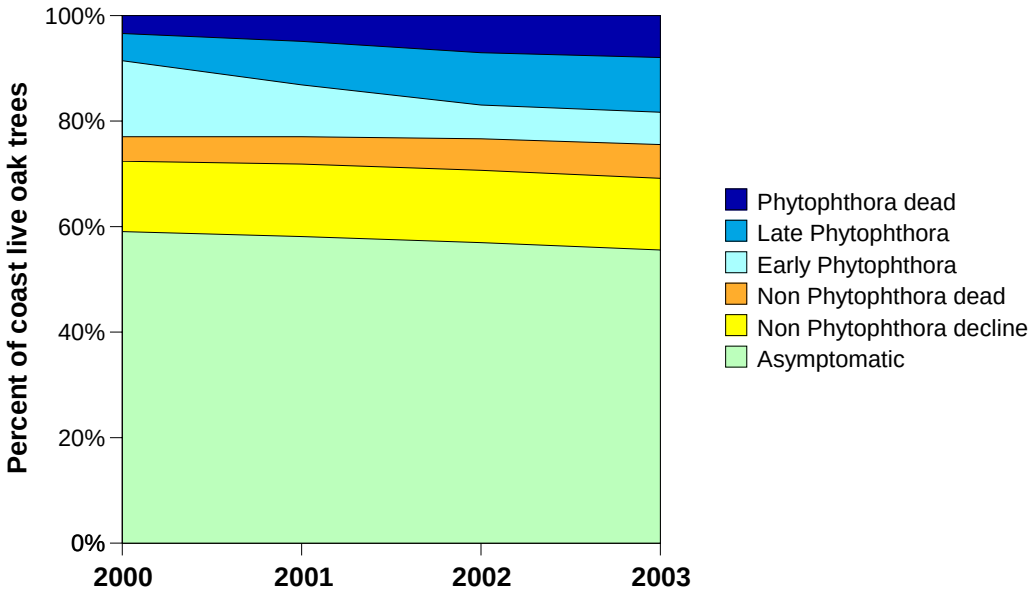

Figure 12. Changes in health of all coast live oak study trees from September 2000 to September 2003. n=655.

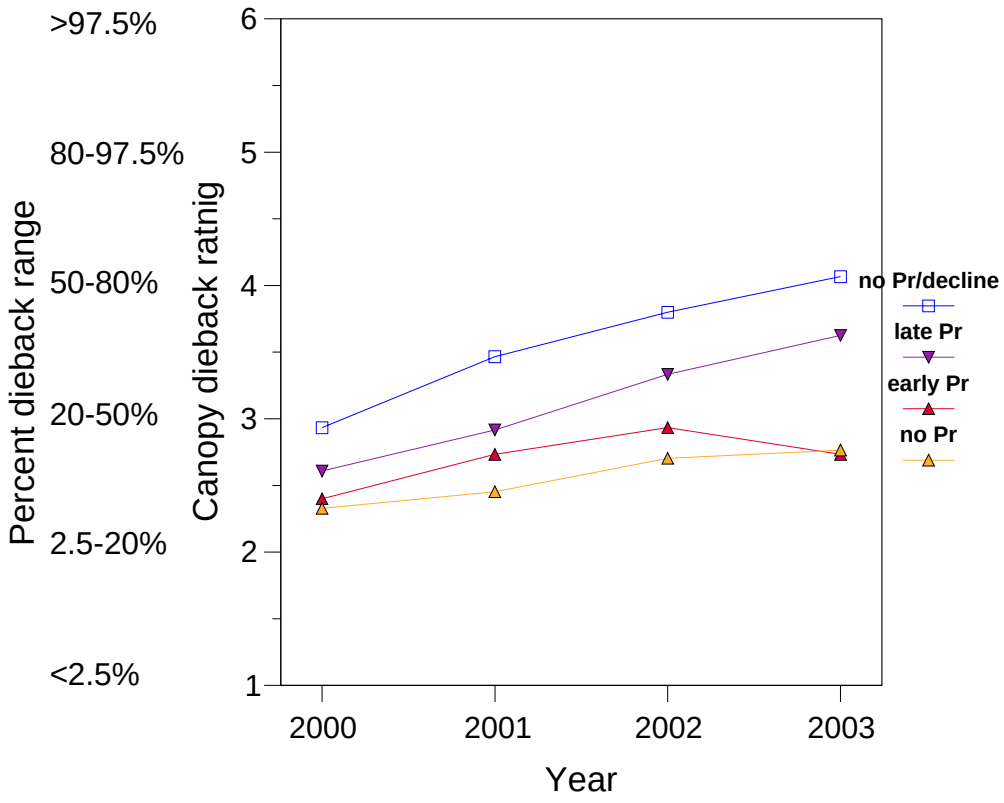

Figure 13. Change in average canopy dieback rating over time for coast live oak subject trees scored in different disease categories in 2003. Pr=*P. ramorum* canker symptoms, decline=severe tree decline due to agents other than *P. ramorum.*

Tanoak

During September 2003 monitoring we isolated from five tanoaks with likely new symptoms of *P. ramorum* infection. Four of these trees yielded positive isolations and one was negative. The new symptoms on the tree in which the isolation was negative were classic symptoms, so we have provisionally scored this tree as positive for *P. ramorum*. Based on September 2003 ratings, disease incidence has increased from 33% in September 2000 to 39% in September 2003, at a roughly constant rate of 2% a year (Figure 14). A comparison of Figures 12 and 14 shows that tanoak continues to develop new *P. ramorum* canker symptoms at a higher rate than coast live oak.

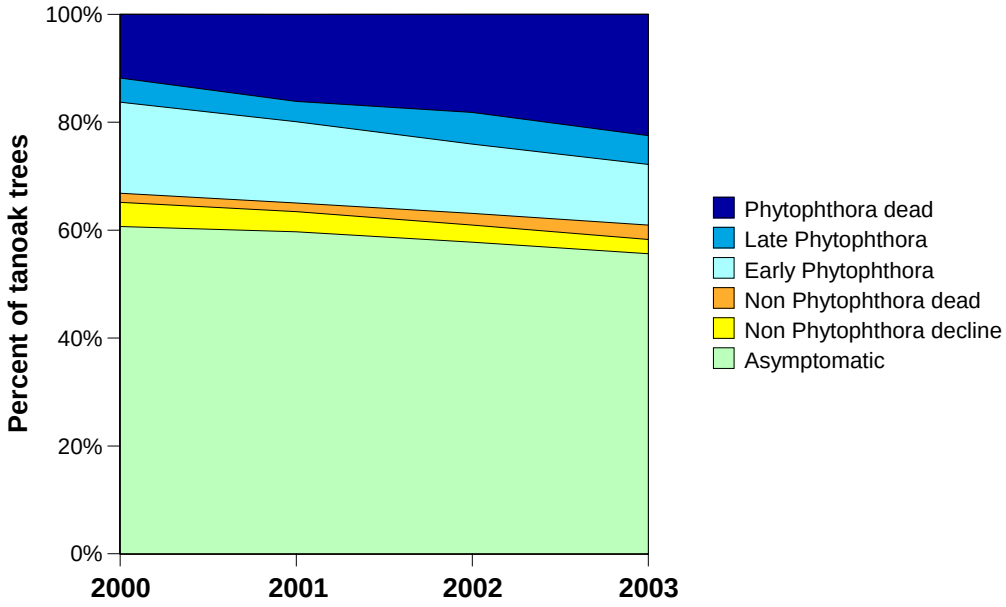

Figure 14. Changes in health of all tanoak study trees from September 2000 to September 2003. n=187.

Figure 15 shows cumulative mortality rates by causal agent for both tanoaks and coast live oaks in the study. All of the tanoaks that have died within plots showed evidence of *P. ramorum* canker. Among coast live oak trees, *P. ramorum* canker has accounted for the majority of the recent mortality, but coast live oak trees have also died from other causes, most commonly infection by decay fungi. A few coast live oaks that have died have had both *P. ramorum* canker and other diseases such as severe canker rot and it has not been possible to unequivocally assign the cause of death to only one of these agents. Tanoak showed large increases in mortality due to *P. ramorum* canker in 2001 and 2003 whereas coast live oak showed the largest increase in mortality in 2002 (Figure 15). This asynchrony in mortality peaks probably reflects the relative speed of symptom progression, which is generally faster in tanoak.

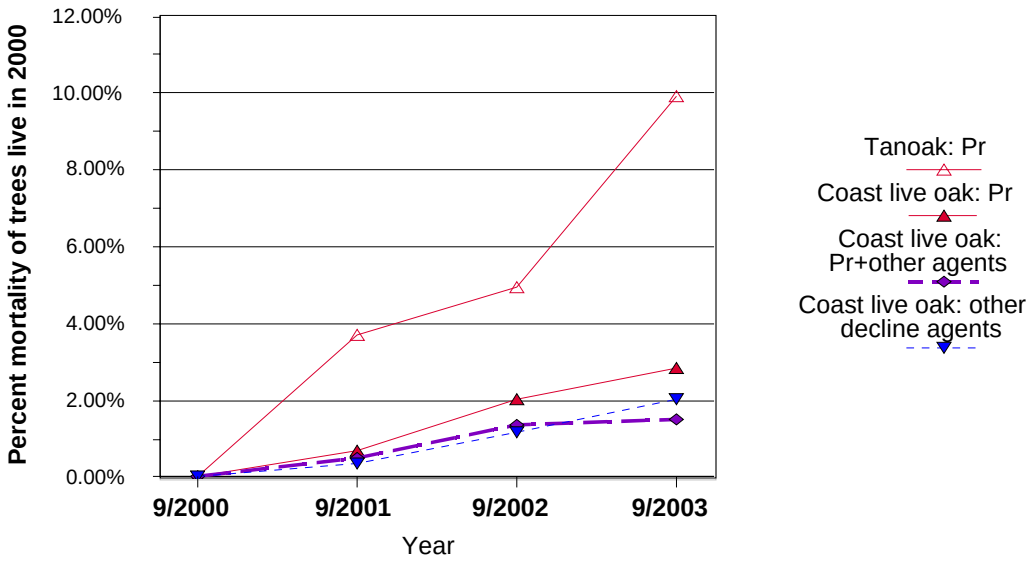

Figure 15. Increase in mortality among tanoak and coast live oak study trees alive in September 2000 due to *P. ramorum* (Pr) and other agents (e.g., wood decay or root rot fungi).

Failures in coast live oak study trees

Over the study period, trees with *P. ramorum* canker symptoms have failed at a significantly higher rate than uninfected trees. The overall failure rate from 2000 through 2003 in trees with *P. ramorum* symptoms was 31.8%, compared with a 2.4% failure rate for trees without *P. ramorum* symptoms. These percentages include all study trees that were live or standing dead in 2000 that subsequently failed at the roots or bole or had a failure of a branch or scaffold at least 20 cm in diameter. Currently, trees with symptoms of *P. ramorum* canker constitute 24% of the coast live oaks in the study but 80% of the coast live oaks with failures.

Trees that had advanced symptoms of disease in 2000 (late or dead) have failed at higher rates over the study period than have other dead and declining trees (Figure 16). About 65% of the trees with late *P. ramorum* canker symptoms in 2000 had failed by 2003. Among trees that were dead as a result of *P. ramorum* canker in 2000, the failure rate was about 73% by 2003. Put another way, the time required for half of the trees to have a failure above the threshold size was 2 years for dead trees with *P. ramorum* symptoms and 2.5 years for trees with late *P. ramorum* canker symptoms.

Only 10.5% of the trees that had only early *P. ramorum* canker symptoms in 2000 had failed by 2003, after three years of observation. Most of the failed trees had progressed to the late or dead *P. ramorum* symptom classes prior to failure, but two trees had only early symptoms of *P. ramorum* at the time of failure (Figure 17). Two asymptomatic trees also failed during the observation interval, both in 2003 (Figure 17). These were both multistemmed sprout-origin trees. One failed at the roots and was a root failure and the other as a root crown failure. Both trees were in relatively good condition prior to failure.

In both trees with and without *P. ramorum* canker symptoms, bole failures were the most common type of failure. Sixty percent of the failures occurring in trees with *P. ramorum* canker symptoms and 58% of failures in trees without *P. ramorum* canker symptoms were bole failures (Figure 18). In contrast, root failures have been relatively uncommon overall. Only one root failure has occurred during the monitoring interval (Figure 18.) Overall, scaffold and branch failures constituted 17% of the failures in asymptomatic trees and 27% of the failures in trees with *P. ramorum* canker symptoms. Given the small number of observed failures in asymptomatic trees (n=12), this difference is not significant. The overall frequencies of various failure types are similar to that observed in our retrospective study of failures

(Swiecki and Bernhardt, 2003a) with the exception that root crown failures appear to be somewhat more common overall in this study than in the retrospective study.

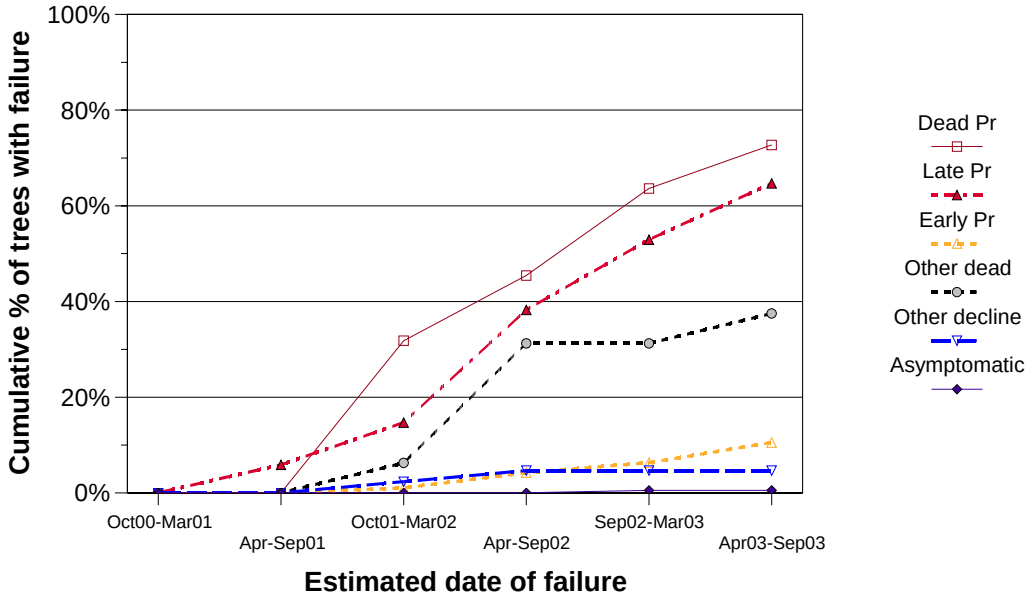

Figure 16. Increase in failures among coast live oak study trees grouped by disease status in September 2000.

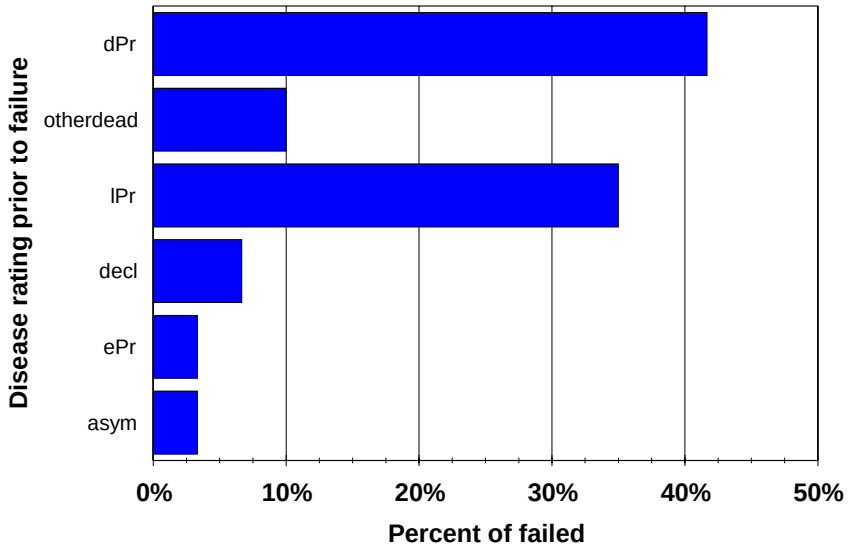

Figure 17. Failures occurring in study trees classified by the most recent disease rating prior to failure. Failures occurred within 12 months of the disease rating. n=60 failed trees.

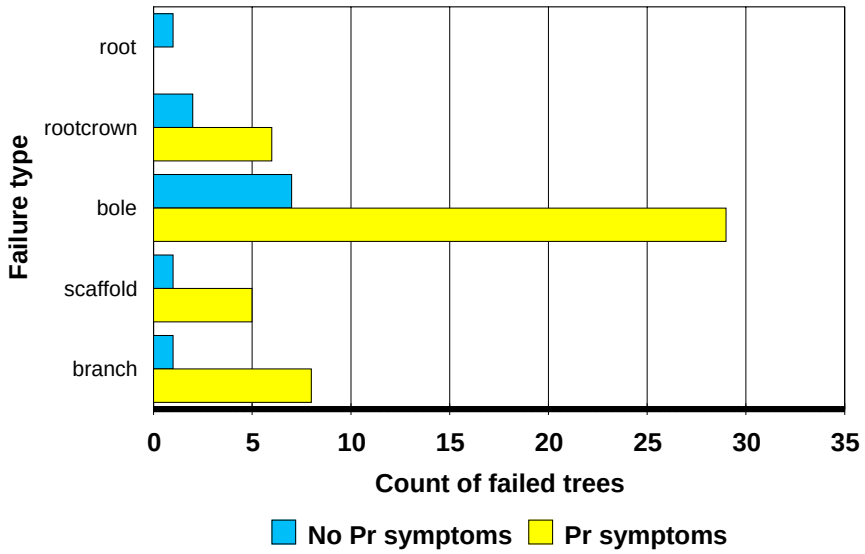

Figure 18. Failure types of coast live oak trees occurring between September 2000 and September 2003. Trees with multiple failures are recorded in the category of the first failure. n= 12 trees without *P. ramorum* symptoms and 48 trees with *P. ramorum* canker.

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