Phytophthora ramorum canker: Factors affecting disease progression and failure potential

2002-2003 Contract Year Annual Report

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Cover photo:
Tree 161, a subject tree near Phoenix Lake, Marin Municipal Water District, September 2002. This coast live oak had early *Phytophthora ramorum* canker symptoms in September 2000, without beetle boring or *Hypoxylon thouarsianum* fruiting, with a 20 to 50% canopy dieback rating. Due to canker rot symptoms, decay impact was rated as moderate in 2000. By September 2001 the canopy dieback rating had increased to 50 to 80%, but beetle boring and *H. thouarsianum* fruiting were still absent. One of the two main stems failed at the root crown in the first half of 2002.

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SUMMARY

This report presents data from the third year of observations in a case-control study to examine the role of water stress and various other factors on the development of *Phytophthora ramorum* stem canker, commonly called sudden oak death, in coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). This study compares subject trees that exhibited symptoms of *P. ramorum* infection (case trees) with symptomless (control) trees. In September 2000, 2001, and 2002 we collected data in 150 circular plots (8 m radius) in areas where disease caused by *P. ramorum* was prevalent. Each plot was centered around a case or control subject tree. Plots were established at 10 locations in Marin County, and 1 location each in Sonoma and Napa Counties.

Among all surveyed study trees (includes trees dead at the start of the study), the apparent infection rate among tanoaks has increased from 33% in September 2000 to 37% in September 2002. During the same time interval, the apparent infection rate among coast live oaks has increased from 19% to 20%. Disease symptoms and tree decline have progressed more rapidly in infected tanoaks than in infected coast live oaks. *Phytophthora*-related mortality has increased from 12% of all monitored tanoak in 2000 to 19% in 2002. During the same period, *Phytophthora*-related mortality among all monitored coast live oak has increased from 5% to 8%.

Between September 2000 and September 2002, failures occurred in 5.6% of the coast live oaks in the plots. Most failures occurred in dead trees, but trees with both *P. ramorum* cankers and evidence of beetle boring and/or sporulation of *H. thouarsianum* (i.e., late disease symptoms) also failed at a high rate over the monitoring period. By September 2002, cumulative failure rates had reached 53% among trees which were dead due to *P. ramorum* infection in September 2000, and 37% among those trees scored as having late symptoms of *P. ramorum* infection in September 2000. Among trees rated as having early symptoms in September 2000 (i.e., bleeding cankers only), 5% had experienced a failure by September 2002. In comparison, 4% of trees rated as in decline, and none of the trees rated as healthy in September 2000 had failed by September 2002. Of the trees with failures, 57% experienced bole failures and 11% experienced root crown failures. The remaining failures occurred in branches and scaffolds.

For coast live oak, overall levels of canopy dieback increased between 2000 and 2002. Canopy dieback levels were highest in trees with late symptoms of *P. ramorum* canker and those in decline due to other reasons. Canopy thinning also increased for trees with symptoms *of P. ramorum* canker. Many coast live oaks with extensive *P. ramorum* cankers that do not completely girdle the trunk have exhibited a chronic slow decline syndrome characterized by progressive canopy dieback and thinning. These symptoms differ from the more acute syndrome ("sudden oak death") in which canker-girdled trees show synchronous necrosis of the canopy without extensive thinning or fine branch dieback occurring beforehand.

To test the hypothesis that *Phytophthora* canker in coast live oak is more likely to occur in trees that are vigorous and/or fast-growing than in trees that are suppressed and/or slow-growing, we measured growth rings in a small sample of increment cores collected in September 2002. Growth increments averaged 2.7 mm/year during the high rainfall years 1994 through 1998. The close spacing and relatively wide width of rays in the wood, vague structural demarcation of annual growth increments, and the presence of false annual increments made the cores extremely difficult to read. Average increment growth did not differ significantly between trees with *P. ramorum* symptoms, trees declining or dead due to other agents, and asymptomatic trees. Given the small sample size and the high amount of variability in the data, this pilot study did not have enough statistical power to detect small to moderate differences in increment growth. On the basis of this limited investigation, we cannot rule out the possibility that differences in increment growth may be related to *P. ramorum* susceptibility.

Average subject tree stem water potential (SWP) readings for 2002 were intermediate between those measured in 2000 and 2001. Year-to-year changes in SWP can be explained by trends in

rainfall totals and potential evapotranspiration. SWP readings for individual trees in all three years were highly correlated. Most trees with symptoms of *P. ramorum* infection showed year-to-year changes in stem water potential readings that were similar to those of asymptomatic trees. Most coast live oaks with late *P. ramorum* canker symptoms have maintained high SWP levels and do not show progressive increases in water stress. We hypothesize that SWP levels in these trees remain high because leaf area loss due to diffuse canopy dieback reduces evapotranspiration while roots continue to function normally.

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 in an early stage.

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 two years of this project have been

reported (Swiecki and Bernhardt 2001a, 2002a, 2002b). 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 paper reports on the third 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

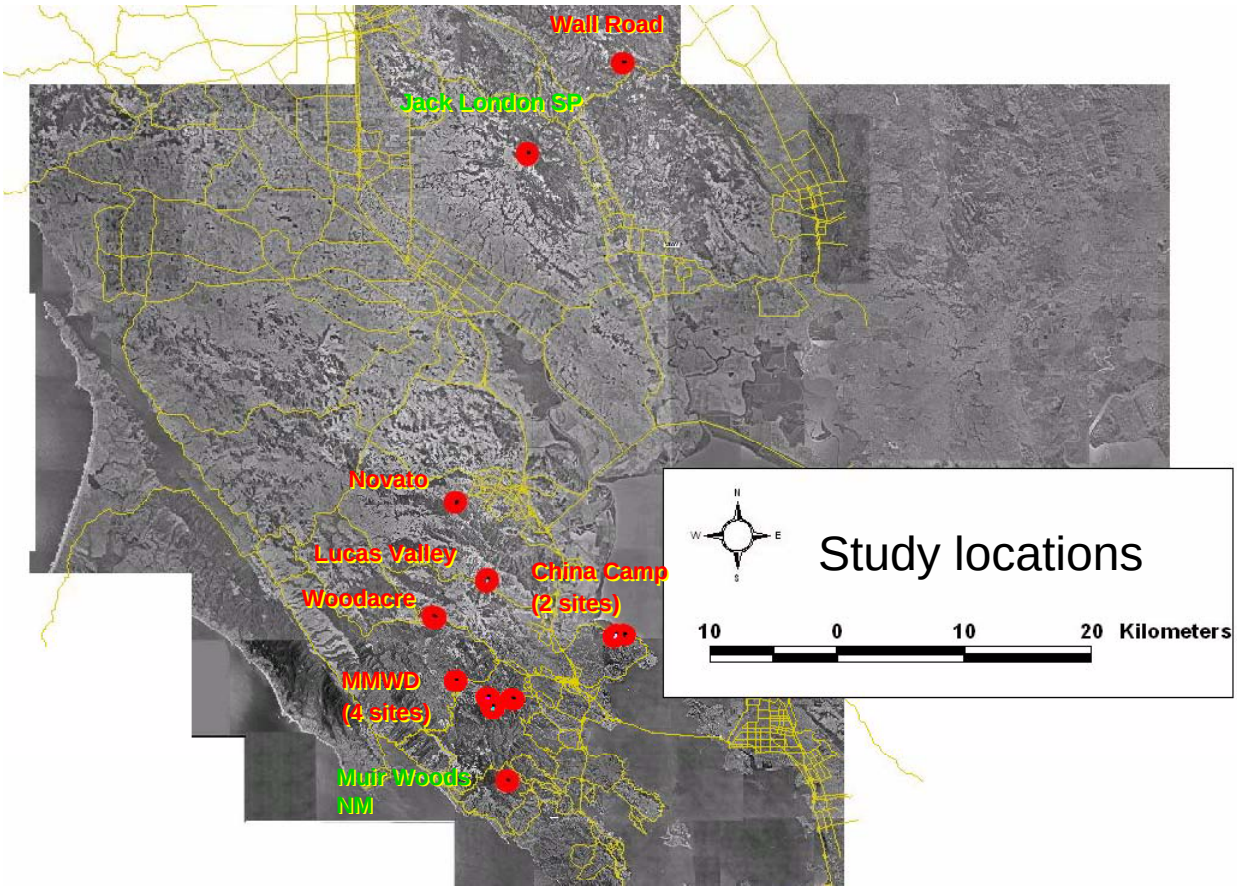

Figure 1. Map showing locations of study areas in Marin, Napa, and Sonoma counties. Background image is a mosaic of USGS digital aerial orthophotos.

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 (2000-2002), 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. In 2001 and 2002, 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:

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

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

Increment cores

In 2002, we collected increment cores from a number of trees to investigate whether recent increment growth is related to disease risk or progress. Because injury associated with the increment borer has the potential to influence further disease progress or susceptibility, cores were taken only from plot trees that were dead at the time of coring. For each plot in which a core was taken, a matched core was collected from a live tree without *P. ramorum* canker symptoms located outside of the plot but otherwise as close to the cored plot tree as possible. Cores were taken from the side of the tree at right angles to trunk lean or sweep. Most cores were collected at a height of 120 to 140 cm, and were placed to avoid irregularities or areas with obvious decay where possible. Cores were 5 mm in diameter and generally included 90 to 100 cm of wood.

Cores were stored frozen at –19 C in a sealed container in plastic straws until mounting to minimize shrinkage, warping, and checking. Cores were glued on grooved wood blocks and sanded with a series of sandpapers, finishing with 400 grit sandpaper. We viewed cores at 14x to 60x magnification using a stereomicroscope to identify growth increments. We measured growth increments with a calibrated ocular reticle at 14x magnification. To adjust for the fact that many

cores were not directly along a radius, increments were measured parallel to the radial rays in the wood. Annual increment measurements were compared with local rainfall data to help identify possible false annual rings.

Additional tree and plot variables

Data collected on subject trees are listed in Table 2. 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.

The disease status of all coast live oak, black oak, and tanoak trees within plots was evaluated in September each year. In 2002, we collected additional information on each plot tree as well as distance/azimuth coordinates from the plot center tree to facilitate continued tracking of disease progress in these trees. Table 2 also lists the variables we evaluated for each plot tree.

Plot-related variables were assessed on an 8 m radius fixed-area plot centered at the subject tree. Table 3 lists the variables we evaluated for each plot. Most plot variables were initially evaluated in 2000, but some additional plot variables were added in 2001 and 2002. 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. We used Dunnett's test to compare dieback ratings of trees in various disease classes against asymptomatic trees.

We also used the recursive partitioning platform in JMP® 5.0.1.2 to develop exploratory models relating various predictor variables to the disease progress outcome. The platform recursively partitions 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. For continuous predictor variables, the partitions are created by a cutting value which divides the sample into values below and above the cutting value. For categorical predictors, the sample is divided into two groups of levels. For the binary categorical outcome disease progress, the estimated probability for each response level is the fitted value, and the most significant split is determined by the largest

likelihood-ratio chi-square statistic. Splitting was done interactively and was stopped when an endpoint had fewer than four trees in it or consisted of all failures or controls. We calculated and compared k-fold crossvalidated G^2 statistics (k = 5) for candidate models to assess relative improvement in fit when building models.

Table 2. Tree variables measured for subject trees, other plot trees, and out of plot trees used for increment coring.

General tree descriptors				
Variable	Trees rated[1]	Year(s) evaluated[2]	Method	Scale/units and notes
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	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
P. ramorum canker-related symptoms				
Phytophthora-related symptoms	S,A,P: C:	2000-on 2002	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.

Table 2. 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)				
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 scale[2] 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.
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	visual estimate	0-2 Scale: (0) none; (1) slight; (2) pronounced
Canopy dieback	S A P,C	2000-on 2001-on 2002-on	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 as 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	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

Table 2. 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				
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.
Defect codes	S,A: P: (if failed)	2002	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= cored trees located beyond plot edges

[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 3. 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 3: 20% to < 50% 6: 97.5% to 100%
1:< 2.5% 4: 50% to < 80%
2: 2.5% to <20% 5: 80% to < 97.5%

[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

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

Comparison of 2000, 2001, and 2002 stem water potentials

Overall, stem water potential (SWP) readings in September 2002 were intermediate between September 2000 and September 2001 SWP readings (Figure 2). Average SWP values for the 10 coast live oak locations across the three years were not significantly correlated with corresponding vapor pressure deficits. Instead, year-to-year changes in SWP can be more readily explained by precipitation and potential evapotranspiration (ETo) in the preceding season(s) (Figure 3).

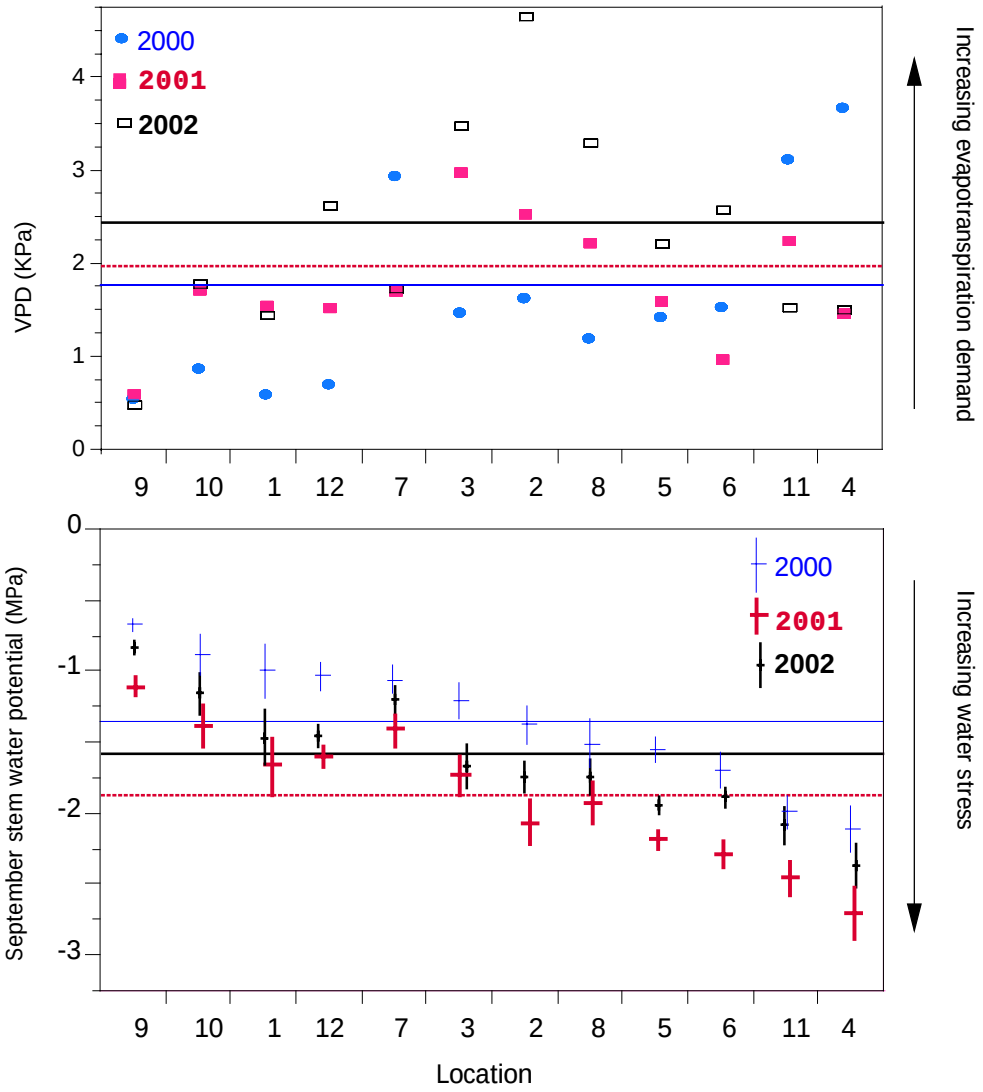

Figure 2. Top graph: Calculated average vapor pressure deficits (VPD) for each location during the period that stem water potentials were measured. Bottom graph: Average stem water potentials for trees at each location in 2000, 2001, and 2002. Vertical lines represent standard error of the mean. For both graphs the overall mean for each year is shown by a horizontal line (dashed blue line=2000, dotted red line=2001, and solid black line =2002). At locations 9 and 12, subject trees are tanoak; at all other locations subject trees are coast live oak.

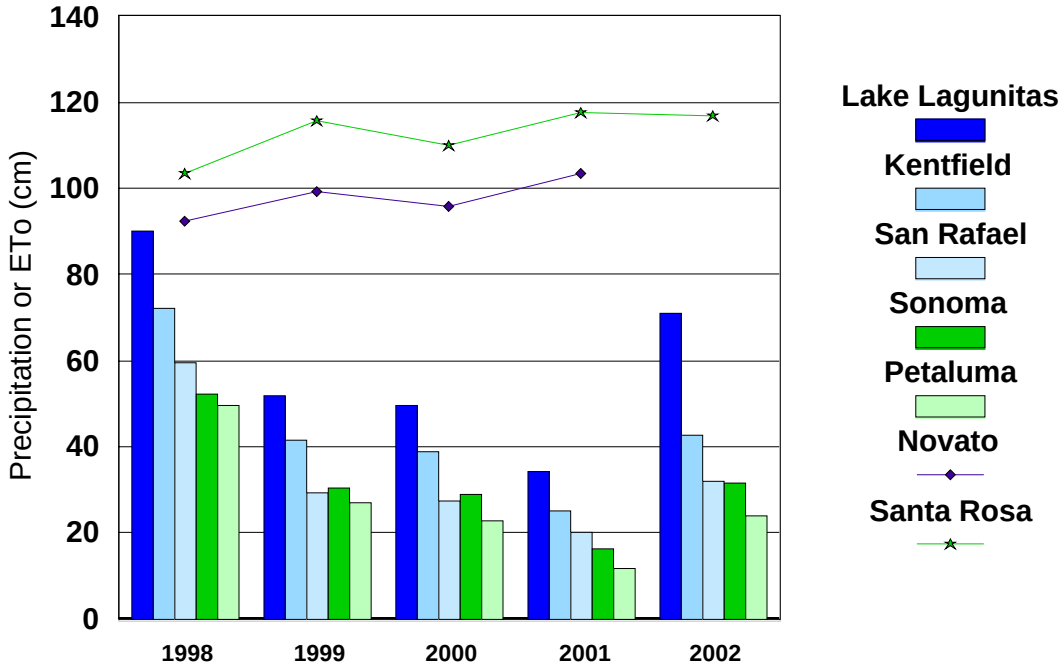

Figure 3. Seasonal rainfall totals (bars) and annual ETo (lines) for weather stations near the study locations in Marin (blue) and Sonoma (green) counties. Rainfall data are from the Western Regional Climate Center database and cover the period from September of the preceding year to August of the year listed. ETo data are from California Irrigation Management Information System (CIMIS) stations and are calculated for the entire calendar year (January-December). ETo data from 2002 for Novato are not available.

The lowest overall SWP readings over the three-year observation period were measured in September 2001 (Figure 2). That year also had the highest cumulative ETo and the lowest precipitation (2000-2001 season) over the observation period. The highest average SWP readings were measured in September 2000, even though rainfall was greater in the 2001-2002 rainy season than in the 1999-2000 season (Figure 3). However, precipitation for the two years preceding the 1999-2000 rainy season was much higher than in the two years preceding 2001-2002 (Figure 3), which could have led to higher soil moisture levels and hence higher SWP levels in 2000 than in 2002. Annual ETo was also lower in 2000 than in 2002, which would have reduced overall water use and contributed to higher SWP levels in 2000.

SWP readings made on the same trees for multiple years are highly correlated with each other (Figure 4). In general, most trees retained their SWP ranking relative to other trees. For example, trees with higher than average SWP in 2000 generally had higher than average SWP in 2001 and 2002. Hence, September SWP readings appear to be a reasonably repeatable indicator of the maximum seasonal water stress that trees at a given site experience.

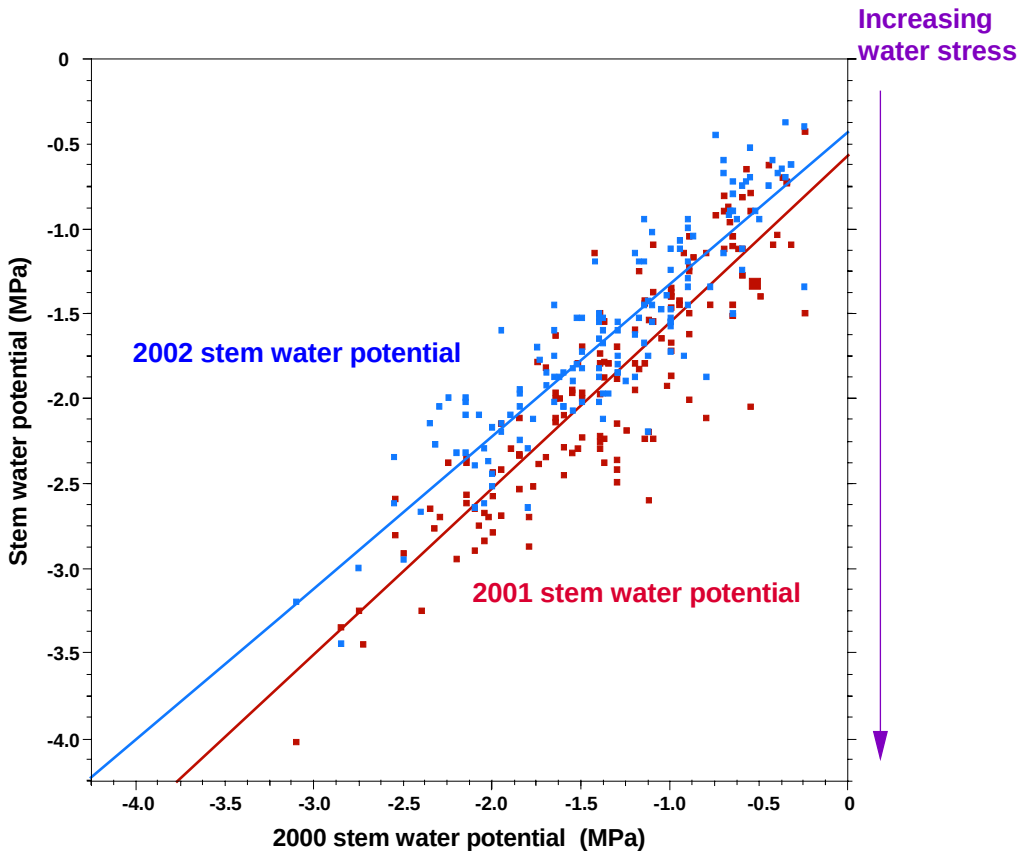

Figure 4. Correlation between 2002 (blue) and 2001 (red) and 2000 stem water potential (SWP) readings.

In 2000 we measured SWP of only the center subject tree (case or control) in each plot. To determine how representative these SWP values were of the tree water status within plots, we took SWP readings from additional plot trees in 35 of the 150 plots (one additional tree per plot except one plot with two additional trees) in 2001 and 2002. Readings between pairs of trees from the same plot were significantly correlated in both years (adjusted $R^2 = 0.577$ and 0.784 in 2001 and 2002, respectively; Figure 5). This suggests that much of the variation in SWP is related to the available soil moisture level within each 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 subject tree or a different tree) provides an indication of tree water stress levels within the plot as a whole. On the basis of this correlation, we have used plot average SWP rather than subject tree SWP as a predictor in some models. This allows us to include SWP readings from 2001 and 2002 as a factor for plots in which the subject tree has died.

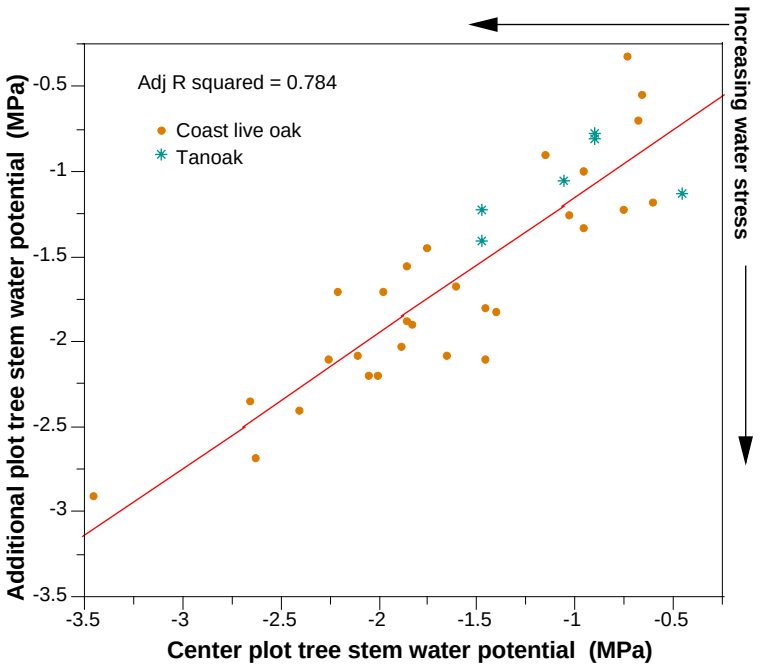

Figure 5. Correlation between stem water potential (SWP) of subject trees and SWP of an additional tree within the same plot, 2002 data. Red dots = coast live oak, green asterisks = tanoak. The regression line is the solid red line.

Relationship between disease progress and stem water potential

Most trees with symptoms of *P. ramorum* infection showed year-to-year changes in stem water potential readings that were similar to those of asymptomatic trees. As discussed above (Figure 2), mean SWP levels of both symptomatic and asymptomatic trees were lowest in September 2001. September 2000 SWP readings were generally higher than September 2002 readings. Overall, 70% of the coast live oak subject trees exhibited this general pattern, which was generally consistent across groups of trees with different sequences of symptom progression (Figure 6).

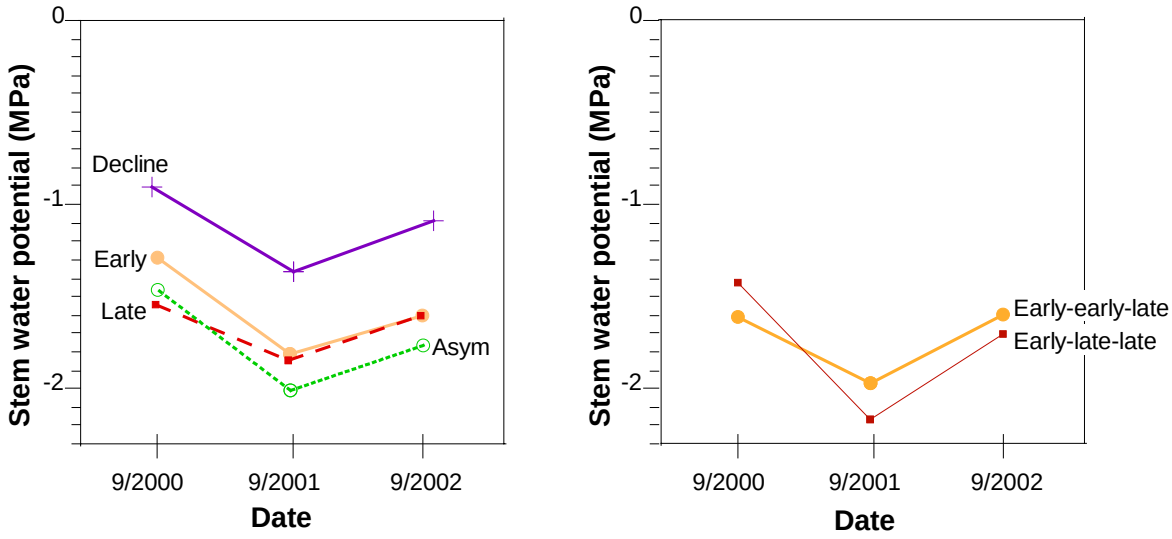

Figure 6. Changes in September stem water potential measurements for coast live oak in six symptom classes. **Left**: trees with the same disease rating on all three observation dates. Early: early symptoms (bleeding cankers only) of *P ramorum* infection (n=19), Late: late symptoms (bleeding cankers plus beetles and/or *H. thouarsianum* fruiting bodies) (n=9), Asym: asymptomatic (n=104). Decline: severe decline (appear likely to die within 10 years) due to factors other than *P. ramorum* (n=17). **Right**: trees showing disease progression during the observation interval. Early-early-late: early symptoms in September 2000 and 2001 and late symptoms in September 2002 (n=9), Early-late-late: early symptoms in September 2000 and late symptoms in September 2001 and September 2002 (n=13). Trees which showed symptoms of severe decline unrelated to *P. ramorum* along with *P ramorum* symptoms are excluded from this data set.

Trees rated as being in severe decline followed the same overall SWP trend, but stand out because they have the highest SWP values overall (Figure 6). Relative to other symptom classes , the decline class also has the highest overall levels of canopy dieback and thinning and the lowest average sky-exposed canopy. Most declining trees that we have observed are in decline due to infection by canker rot fungi; many are also highly suppressed trees. These are trees that presumably have functional root systems but have declining tops with reduced leaf area. Hence these trees tend to have high SWP readings (low water stress).

Among 17 trees that were rated with late symptoms of *P. ramorum* infection in both 2001 and 2002, 6 (35%) had higher SWP in 2002 than in 2000. This is a significantly greater proportion (Fisher's exact test $p = 0.0074$) than among the remaining symptom classes, in which only 8.8% (10/103) had higher SWP in 2002 than in 2000. We surmise that SWP is high in many of the trees with late symptoms because roots continue to function while total leaf area and hence evapotranspiration (ET) is decreased due to diffuse canopy dieback (discussed below).

Factors associated with SWP

We used regression analysis to evaluate whether tree and plot factors were related to the variation in SWP. Although the range of SWP values recorded for tanoak fell completely within the range of coast live oak SWP values, average SWP of tanoak was significantly higher than that of coast live oak (t-test $p < 0.0001$ for 2002, 2001, and 2000). Average SWP readings for tanoak and coast live oak differed by 0.71 MPa in 2000, 0.76 MPa in 2001, and 0.58 MPa in 2002. Because of this consistent difference, we constructed separate SWP models for coast live oak and tanoak.

The analyses include both subject trees and additional trees within plots for which SWP readings were made in 2001 and 2002.

Coast live oak

Regression models for 2002 SWP readings accounted for about two-fifths of the variation in SWP (Table 4). As observed in analyses of 2000 and 2001 data, SKY-EXPOSED CANOPY, ANNUAL INSOLATION, and *PHYTOPHTHORA* GIRDLING RANK were significant terms in the model. The negative correlations between SWP and both SKY-EXPOSED CANOPY and ANNUAL INSOLATION are consistent with expectations based on standard plant water relations models. Trees with greater solar exposure, due to either canopy position or slope / aspect combination, experience greater seasonal evapotranspiration demand than trees with less solar exposure. By the end of the summer, trees with greater solar exposure should therefore have lower SWP values (i.e., elevated levels of water stress).

SWP was also correlated with variables related to *P. ramorum* infection (Table 4). As observed in 2000 and 2001, other variables related to *P. ramorum* infection could be substituted into the regression model in place of *PHYTOPHTHORA* GIRDLING RANK, although these other variables decrease model fit somewhat. These variables include *PHYTOPHTHORA* CLASS (asymptomatic, early, late), *PHYTOPHTHORA* CANKER COUNT, CANOPY DIEBACK, and CASE 2002 (i.e., the binary disease presence variable). For all of these disease variables, *Phytophthora* canker symptoms were positively associated with higher SWP readings, i.e., disease was more common in trees that exhibited relatively low levels of water stress. The positive association between disease and high SWP can be interpreted to indicate that trees located in relatively moist areas are at higher risk for disease than trees located in drier sites.

DECAY IMPACT was significant in the 2000 and 2002 models and could not be replaced in the model by the CANOPY DIEBACK variable. However, it could be replaced by the THINNING variable without affecting model fit. We have previously suggested that the association between DECAY IMPACT and SWP could indicate that moist sites favor the development of wood decay through effects on the host and/or the wood decay fungi involved. An alternate explanation is that SWP is elevated in trees with extensive decay simply because the canopies of these trees are thinning, leading to reduced leaf area and lower evapotranspiration demand.

Table 4. Regression model for midday stem water potential (MPa) of coast live oak in September 2002.

Source	DF	F Ratio	Prob>F	Adjusted R^2	n
Overall model	4	28.41	<0.0001	0.41	160
Model terms	**DF**	**F Ratio**	**Prob>F**	**Parameter estimate**	
Sky exposed canopy	1	51.31	<.0001	-0.171	
Annual insolation (MJ/m^2)	1	8.52	0.0040	-0.000132	
Phytophthora girdling rank	1	25.66	<.0001	0.111	
Decay impact rating	1	8.52	0.0040	0.130	
Intercept				-0.584	

VPD was a significant predictor of coast live oak SWP in 2000, but only for trees with more than 50% sky-exposed canopy (Swiecki and Bernhardt 2001a). VPD was not significantly correlated with 2001 or 2002 coast live oak SWP readings (Figure 2). A lack of correlation between VPD and midday SWP would be expected if water stress was severe enough that most stomata were closed midday irrespective of VPD.

On 8/17/2000, we measured both stomatal conductance and SWP for 7 coast live oaks at location 3. These trees had an overall average SWP of -1.45 MPa and relatively low stomatal

conductance readings that averaged a 84.5 mmol/s/m^2. We would expect that midday stomatal closure is likely to be even more pronounced in September than in mid August due to further depletion of available soil moisture. Therefore, it is plausible the that lack of a relationship between VPD and SWP is largely be due to nearly complete midday stomatal closure throughout the period when readings were made.

Tanoak

There were only two locations (locations 9 and 12) with tanoak subject trees in the study. The small sample size (n = 29 tanoak with SWP readings) limited our ability to fit predictors in the model. As seen in previous years, location was the best predictor for midday SWP in the analysis of covariance model for tanoak, indicating the 2 locations differed in their overall SWP (Figure 2). Although plot annual insolation was significant in the tanoak SWP regression model in 2001 (Swiecki and Bernhardt, 2002), it was not significant in 2002. Variables related to *Phytophthora* canker symptoms were not correlated with SWP in tanoak.

Disease and disease progress in study trees

In September 2000, we made detailed observations of disease symptoms in all of the 150 subject trees (cases and controls) in the study. By reassessing disease symptoms in these trees in September 2001 and 2002, we were able to determine how symptoms progressed during the intervening years and how many previously asymptomatic trees developed *Phytophthora* canker symptoms. Subject trees include 128 coast live oaks and 22 tanoaks. We also rated the overall disease status of all other tanoak, black oak, and coast live oak trees in the plots. The plot trees were classified into the following symptom classes:

- asymptomatic;
- early symptoms of *P. ramorum* infection (bleeding cankers only);
- late *P. ramorum* symptoms (bleeding cankers plus beetle infestation and or *H. thouarsianum* fruiting bodies);
- dead due to *P. ramorum* infection;
- in decline due to agents other than *P. ramorum;* and
- dead due to wood decay fungi.

Decline due to other agents and *P. ramorum* canker symptoms were rated separately, so combinations between *P. ramorum* canker symptoms and decline due to other agents were possible. However, trees with symptoms in both categories constituted less than 2% of all tanoak and 4% of all coast live oak.

Although bleeding cankers caused by *P. ramorum* are often quite distinctive, other agents can also cause at least superficially similar bleeding cankers in oaks and tanoak. In order to determine whether *P. ramorum* or other *Phytophthora* spp. were associated with various atypical cankers, we excised tissue pieces from cankers on 9 coast live oaks and one tanoak and inserted them into PARP media in September and October 2002. We recovered *P. ramorum* from the tanoak sample and one of the coast live oaks. No other *Phytophthora* spp. were recovered from these samples.

Because *P. ramorum* can be difficult to isolate from canker tissue, especially in late summer (D. Rizzo, personal communication), negative isolations do not conclusively prove that *P. ramorum* is absent. We therefore relied on other canker characteristics in order to assign the disease status for the 8 trees from which no *P. ramorum* was isolated. For example, trees colonized by canker rot fungi, *Armillaria* spp., or other wood decay fungi sometimes have bleeding cankers, but these differ in appearance from *P. ramorum* cankers and are normally closely associated with wood decay. We have also seen small, slightly bleeding cankers on small diameter coast live oak stems in some locations. Canker morphology differs from that seen in *P. ramorum* cankers and we have not isolated any *Phytophthora* sp. from this sort of canker.

Based on observations, five of the negative isolations were classified as asymptomatic or in decline; three were classified as having early symptoms of *P. ramorum* infection. Further observations and isolations should help to clarify the disease status of these trees in subsequent years. In addition, two trees originally classified as having early symptoms of *P. ramorum* infection in 2000 had no evidence of the small bleeding cankers upon which the original diagnosis was based Pending further observations which may be more conclusive, we have reassigned these trees to the asymptomatic category.

The proportions of trees in each disease category at each reading date are shown in Figures 7 and 8. Between September 2000 and September 2002, disease symptoms have progressed in the majority of trees that were initially symptomatic (Figures 7, 8). However, the overall incidence of trees with *P. ramorum* canker symptoms was virtually unchanged for coast live oaks and showed only a very slight increase for tanoaks. If the period between infection and external symptom expression can be as long as several years, it is possible that no new infections have been initiated in the tree population since the start of the study.

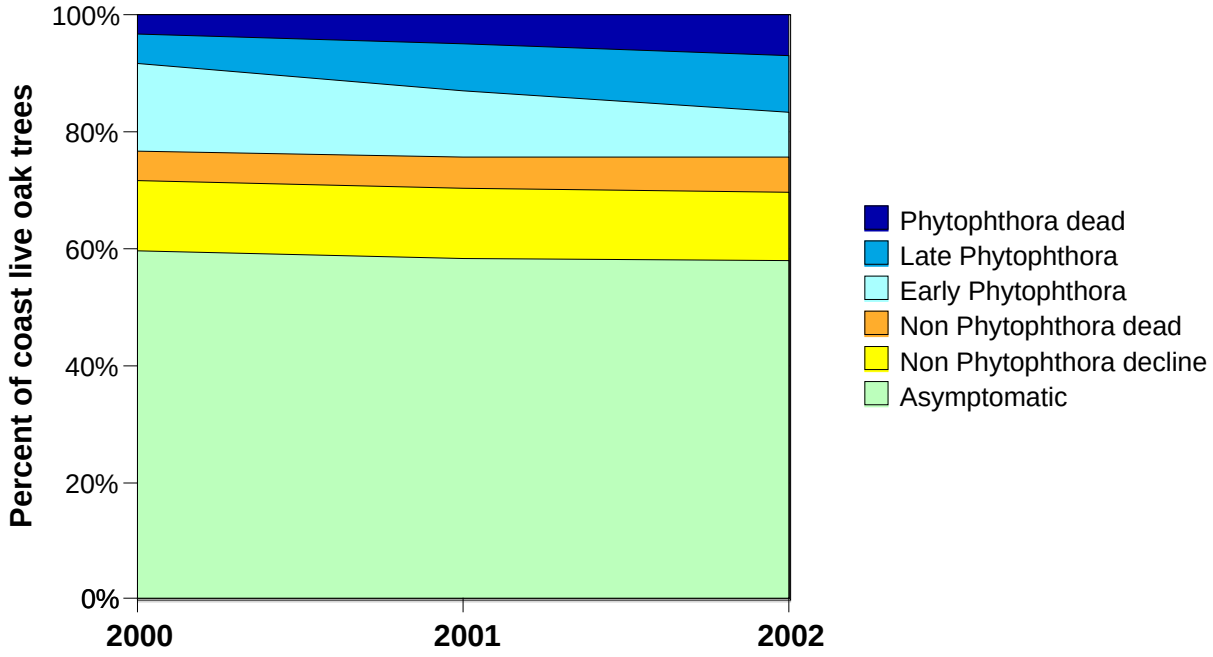

Figure 7. Changes in health of all coast live oak study trees from September 2000 to September 2002. n=654.

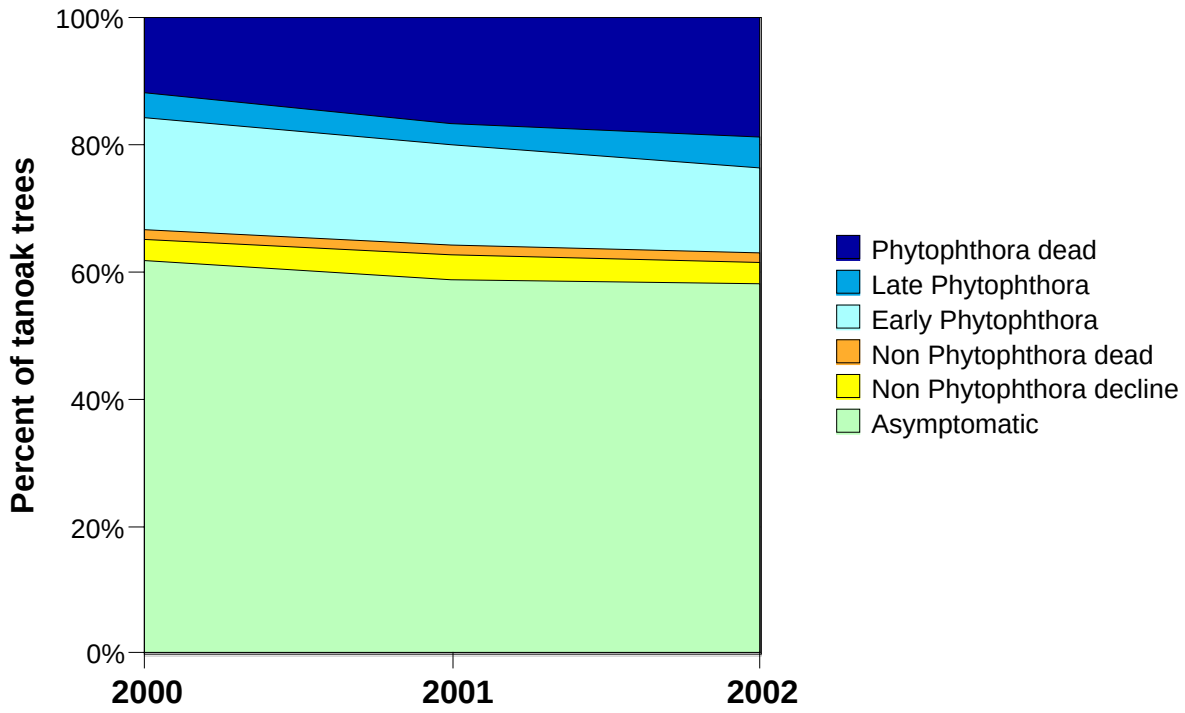

Figure 8. Changes in health of all tanoak study trees from September 2000 to September 2002. n=184.

The incidences of apparent infection and mortality due to *P. ramorum* have been consistently higher in tanoak than coast live oak. The apparent infection rate among tanoak has increased from 33% in September 2000 to 37% in September 2002, whereas in coast live oak, the apparent *P. ramorum* infection rate has increased from 19% to 20%. Overall mortality rates for coast live oak and tanoak are shown in Figure 9. *Phytophthora*-related mortality has increased from 12% of all monitored tanoak in 2000 to 19% in 2002, compared to an increase from 5% to 8% among all monitored coast live oak over this period. Since the study was initiated, all observed mortality of tanoak in study plots has been attributed to *P. ramorum*. However, some coast live oaks have died from other causes, most commonly disease caused by canker rot fungi and other wood decay fungi (Figure 9). Severe decline associated with wood decay fungi has been much more common in coast live oak than in tanoak (Figures 7, 8).

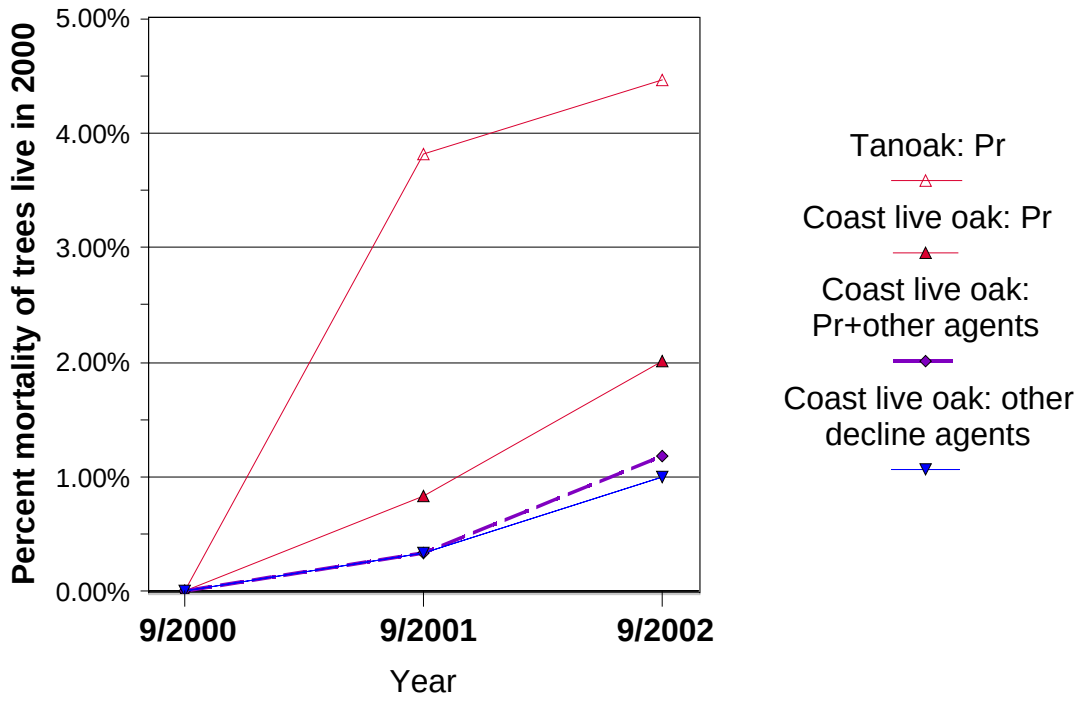

Figure 9. Increase in mortality among tanoak and coast live oak due to *P. ramorum* (Pr) and other agents, most commonly wood decay fungi, that cause tree decline. Both plot and subject trees are included in the percentages.

Coast live oak

We have detailed condition data on the center (subject) tree in each plot. For coast live oak, this is a sample of 128 trees. Based on increased severity of dieback, thinning, and/or decay impact ratings, 26% of the uninfected coast live oak trees showed a decline in overall condition in the absence of *P. ramorum* canker symptoms between September 2000 and September 2002. By comparison, the overall condition of 73% of the trees infected with *P. ramorum* showed a decline in condition during this period.

We used a repeated measures analysis of variance to investigate changes in canopy dieback rating in subject trees that were still live in 2002. In this analysis, the overall effect of time was significant ($p < 0.0001$), but interactions between time and other factors in the model were not significant. As seen in Figure 10, overall dieback ratings increased slightly over time for trees in all disease classes. While increased dieback in diseased trees is understandable, the increased dieback in otherwise asymptomatic trees was not expected. Several factors may contribute to this increased dieback, including several years of relatively low rainfall and shading out of lower canopy branches in dense woodlands. In addition, the overall condition of the trees categorized as asymptomatic ranges from good to poor. A decline rating is assigned only if condition is poor enough that trees are likely to die within a decade. Hence, the asymptomatic condition class includes some trees in poor condition that may be declining, but do not yet merit a "decline" rating.

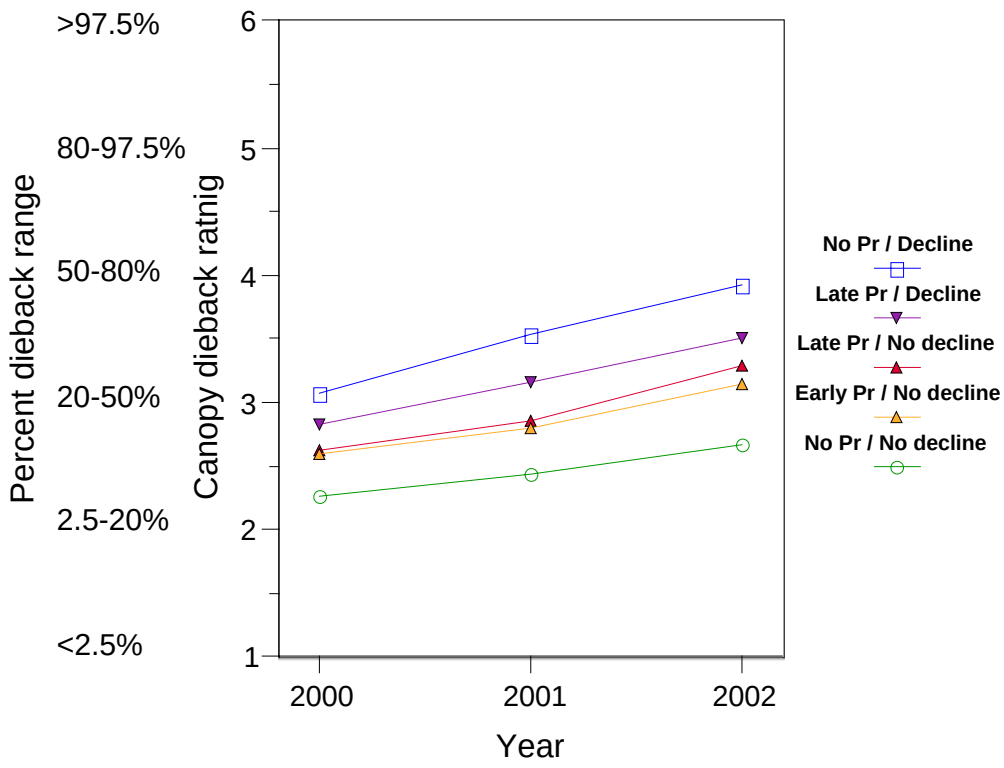

Figure 10. Average canopy dieback rating and disease status in 2001 for coast live oak subject trees (n=123).

In the repeated measures model, *P. ramorum* canker presence ($p = 0.007$), decline due to other agents ($p < 0.0001$), and the interaction between these two factors ($p = 0.0156$) were significant predictors of dieback. Over all three years, dieback was greatest for trees assigned to the decline category, which are trees in poor condition (appear likely to die within 10 years) that show no evidence of *P. ramorum* canker. Most of these trees have high amounts of wood decay, sometimes including large open decay columns (Figure 11), and have been declining slowly for many years. Likewise, trees with symptoms of *P. ramorum* canker had higher levels of dieback than non-declining, asymptomatic trees (Figure 10). The levels of dieback in 2002 were significantly higher for all four classes of diseased trees compared with non-declining, asymptomatic trees according to Dunnett's test.

We observed that many trees with *P. ramorum* canker showed obvious increases in canopy dieback between 2000 and 2002. In addition, canopy thinning ratings increased for 12 of 53 (19%) live trees with *P. ramorum* canker between 2001 and 2002. By comparison, thinning ratings increased for 1 of 20 trees (5%) rated as in decline and 10 of 88 (11%) asymptomatic trees. The overall proportion of trees showing increased thinning over this interval was significantly higher for trees with *P. ramorum* canker than those without *P. ramorum* (likelihood ratio test $p = 0.0389$).

These observations indicate that many trees with extensive girdling by *P. ramorum* canker have developed chronic decline symptoms, which include diffuse canopy dieback, defoliation, and canopy thinning (Figure 12). These symptoms are similar to the type of dieback seen in trees affected by wood decay fungi. This suite of symptoms differs from the rapid foliar necrosis of the entire canopy that has occurred in many trees with *P. ramorum* canker that was the basis for the common name "sudden oak death".

Figure 11. Coast live oak plot tree at location 5 (Miwok Meadows, China Camp State Park) with an extensive open decay column. The tree has no symptoms of *P. ramorum* canker.

Figure 12. *P. ramorum*-infected coast live oak plot tree at location 4 (Phoenix Lake, Marin Municipal Water District) showing canopy thinning and dieback of fine branches throughout the canopy. The tree had bleeding cankers only in 2000, but beetle boring and *H. thouarsianum* sporulation were present in 2001 and 2002. In 2002, girdling by *P. ramorum* cankers was somewhat less than 50%.

Factors associated with disease occurrence

As shown in Figures 7 and 8, there have been few new infections among tanoaks and coast live oaks. The logistic regression models presented in our earlier reports (Swiecki and Bernhardt 2001a, 2002a) showed that various plot and tree factors were associated with disease in the subject tree. For coast live oak, plot variables that were positively correlated with disease included the

count of California bay (*Umbellularia californica*) trees in the plot, the number of plot trees with *Phytophthora* canker symptoms, and the presence of poison oak (*Toxicodendron diversilobum*) in the plot. Tree characteristics that were associated with disease included multiple stems, large total stem cross-sectional area, high levels of canopy exposure, and high stem water potential (SWP). In addition to these factors, logistic regression models based on plot trees other than the subject trees showed a negative association between *Phytophthora* canker symptoms and decline symptoms associated with agents other than *Phytophthora*.

During the September 2002 survey, we attempted to further refine our models by quantifying California bay and poison oak cover in each plot using the 0 to 6 scale (Table 3). The California bay cover variable was significant when substituted for the count of California bay in the plot in the logistic regression models. However, the bay cover variable did not improve overall model fit and was not as highly significant as the count of bay trees in the plot.

The poison oak cover variable was nonsignificant in the models. We detected poison oak in more plots in 2002 than we had in 2001. Many plots contain only scattered, short (<15 cm tall) poison oak plants. These are commonly defoliated in September due to drought stress. Higher rainfall in 2002 could account for the better detection in that year. Alternatively, some new detections of poison oak could be due to the establishment of new poison oak plants from seed.

Factors associated with disease progress

Disease progress can be measured in several different ways. If disease progress is based on trees that advanced between the symptom classes asymptomatic, early, late, and dead, about half (25/52) of the subject trees showed disease progress between September 2000 and September 2002. If disease progress is defined to include expansion of cankers and/or increased levels of canopy dieback or thinning, disease progressed in nearly three-quarters (38/52) of the subject trees between 2000 and 2002.

If we use the first definition, there are enough trees in each outcome class to construct a preliminary disease progress model. We used recursive partitioning to develop the model, to take advantage of the algorithm's ability to assign cut points in continuous variables. In the model we developed, the first split was made on the basis of tree cross sectional area. None of the six smallest trees (cross sectional area <398 cm^2, effective diameter $<$ 22.5 cm) had exhibited disease progress. The partition with larger trees (≥ 398 cm^2 cross sectional area) was next split on the basis of plot canopy cover. Most (11/13) trees in plots with $\geq 97.5\%$ canopy cover showed disease progress. A further partition shows that most of these (9/11) were in plots with at least 20% bay cover. This model points out certain subgroups in which disease progress is unlikely (very small trees) or likely (larger trees in heavily canopied plots with substantial amounts of bay). However, most trees (37/52) do not fit in these particular groups. Disease progress occurred in nearly half (16/37) of these trees, but subsequent attempts to partition these trees resulted in subgroups below our minimum size of 4. Hence, we have not identified any single factor or combination that strongly predicts disease progress between 2000 and 2002.

Although the sample size for tanoak is small, we detected a positive association between disease progress and tree size in these trees. Disease progress was positively correlated with stem cross sectional area (likelihood ratio test $p=0.0289$).

Failures in coast live oak study trees

During plot ratings in September 2000 and 2001, we noted the presence of standing dead and failed trees within plots. Starting in 2002, we recorded more detailed data on all failures of main stems greater than 15 cm DBH and branch failures of at least 20 cm within plots. For trees with multiple failures, only the earliest failure above the size threshold was included in the analysis. If multiple failures occurred in the same time interval, the most severe failure was used.

Between September 2000 and September 2002, failures occurred in 35 of 630 (5.6%) coast live oak trees that were standing in study plots in September 2000. The majority of the observed failures were bole failures (Table 5).

Table 5. Counts of coast live oak trees with failures occurring between September 2000 and September 2002. Trees with multiple failures are recorded in the category of the most severe failure. n=630 trees.

Type of failure	Number of failed trees	Percent of observed failures
Bole ≥15 cm diameter	20	57
Root crown	4	11
Scaffold	4	11
Branch ≥20cm diameter	7	20

Since monitoring began in September 2000, no failures have occurred in asymptomatic trees, i.e., trees lacking symptoms of *P. ramorum* infection or decline due to other agents. Most failures occurred in dead trees or those with late symptoms of *P. ramorum* infection (Figure 13). Fifteen of the 35 trees that experienced failures in 2001 and 2002 were dead prior to failure. In an additional 13 trees, the part of the tree that failed was dead prior to failure. A quarter of the bole and root crown failures (6 of 24) occurred in trees that had green foliage. Five of these six live stem failures occurred in trees with late symptoms of *P. ramorum* infection; the remaining failure occurred in a tree with severe canker rot symptoms. Only one failure occurred in a tree whose most recent disease rating was of early *P. ramorum* symptoms (bleeding cankers without beetle infestation or fruit bodies of wood decay fungi, cover photo). The failed stem of this tree had extensive wood decay affecting more than 75% of the of the stem cross-sectional area.

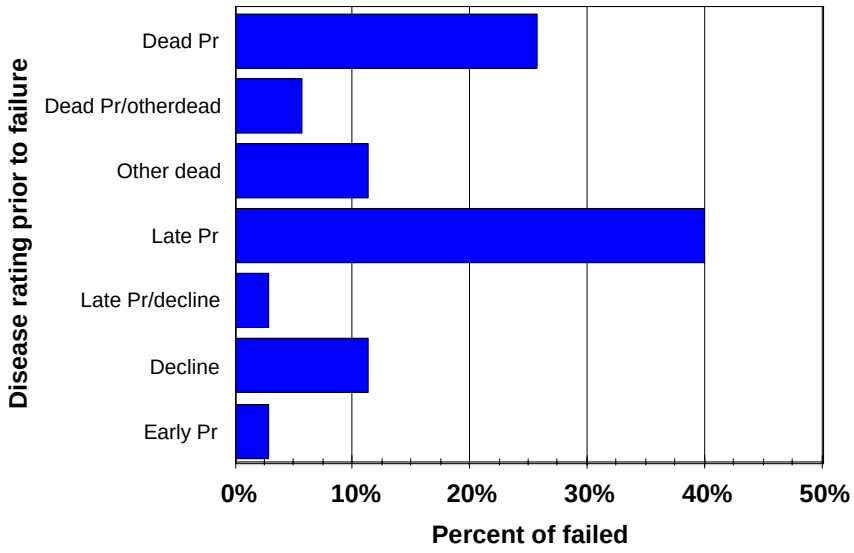

Figure 13. Failures occurring in study trees classified by the most recent disease rating prior to failure. Failures occurred within 12 months of the disease rating.

Cumulative failure rates among trees in different symptom classes in September 2000 are shown in Figure 14. Trees that were dead as a result of *P. ramorum* infection in September 2000 had the highest rate of failure. More than half of these dead trees failed in some way within the next two years, with the greatest percentage of failures occurring between the September 2001 and

September 2002 disease ratings (Figure 14). Trees with late symptoms of *P. ramorum* infection in September 2000 also had a high failure rate, with most failures occurring in the April to September 2002 period. About 40% of these trees had died before they failed (Figure 13). Trees with early *P. ramorum* symptoms in September 2000 or in decline showed low rates of failure over the observation interval (Figure 14). As noted above, no failures occurred in asymptomatic trees over the interval.

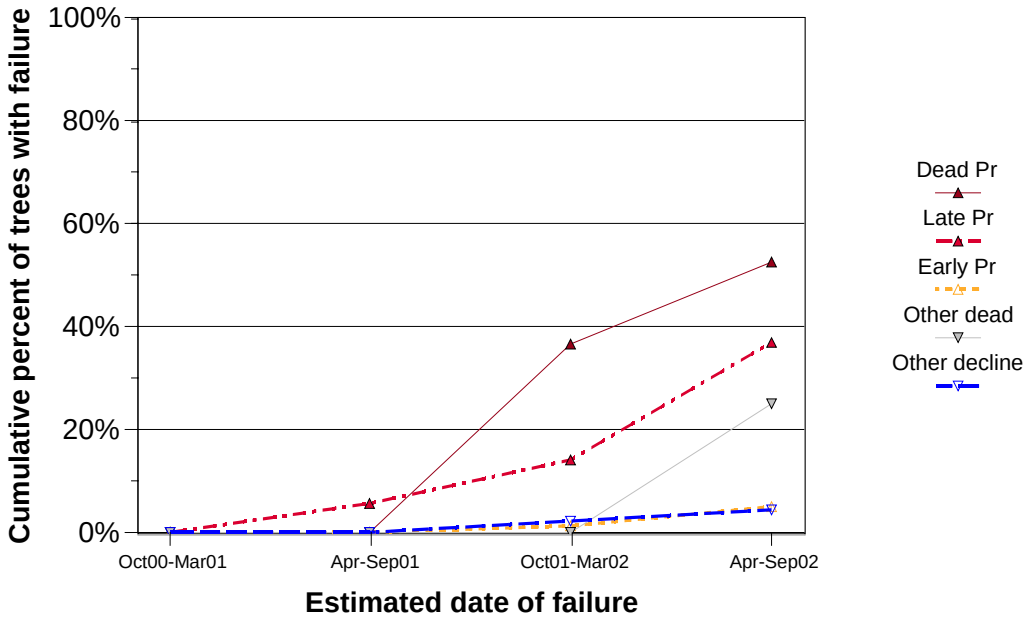

Figure 14. Cumulative failure rates of trees by disease category in September 2000. No failures occurred among trees that were asymptomatic in September 2000. Trees in the 'Decline' category were deemed likely to die within 10 years due to poor condition. Trees in the 'Other dead' and 'Dead Pr' categories had been dead for less than 10 years in September 2000. One tree coded as dead Pr/ other dead was grouped with the dead Pr category.

Increment coring of stems

Based on relationships between disease and several different variables (Swiecki and Bernhardt 2001, 2002), we hypothesized that *P. ramorum* canker in coast live oak might be more likely to occur in trees that are vigorous and/or fast-growing (i.e., larger, more dominant, less water-stressed, not in decline due to other agents) than in trees that are suppressed and/or slow-growing. To test this hypothesis directly, we collected 71 increment cores and disks from recently killed trees within plots and the closest nonsymptomatic tree located beyond the plot. We did not core live trees within the plots to avoid any possibility that the wound made by coring would affect subsequent disease progress or susceptibility. As a pilot study, we processed and made increment counts on 20 of these cores and disks (disks were taken from failed trees) which were sampled from trees at five study locations. We measured at least 20 of the most recent increments, but only the aggregate increment growth for the 5-year period 1994 through 1998 was used for analysis. We assumed that the *P. ramorum* infections in these trees were probably initiated during the last high rainfall period, which ended in 1998, so growth during this period would be the most relevant.

Increment measurements could not be made on many of the cores from dead trees due to high amounts of decay. Increment measurements and counts on sound cores also proved to be difficult due to several factors, including closely spaced and relatively wide rays in the wood, vague structural demarcation of increments, and the presence of false annual increments (Figure 15).

Individual growth increments in the cored trees were also generally small, averaging 2.7 mm/year during the years 1994 through 1998 for the 20 measured trees. This was a period of relatively high rainfall that had some of the largest annual increments in the core samples.

For the 20 trees, we did not detect a significant relationship between the 5-year increment width and DBH (Figure 16), but few large diameter trees (over 60 cm DBH) were included in the sample. Average increment growth did not differ significantly between trees with *P. ramorum* symptoms, trees declining or dead due to other agents, and asymptomatic trees. Given the high amount of variability in the increment widths (Figure 16), a much larger sample than was used in this pilot study would be needed to detect even moderately large differences. Based on this limited sample, we cannot definitively prove or disprove that hypothesis that differences in increment growth may be related to *P. ramorum* susceptibility.

Figure 15. Increment cores collected at DBH for coast live oak uninfected (top three cores) and killed due to *P. ramorum* infection (bottom two cores). Bark side of core is to left for all cores, cm rule is at top.

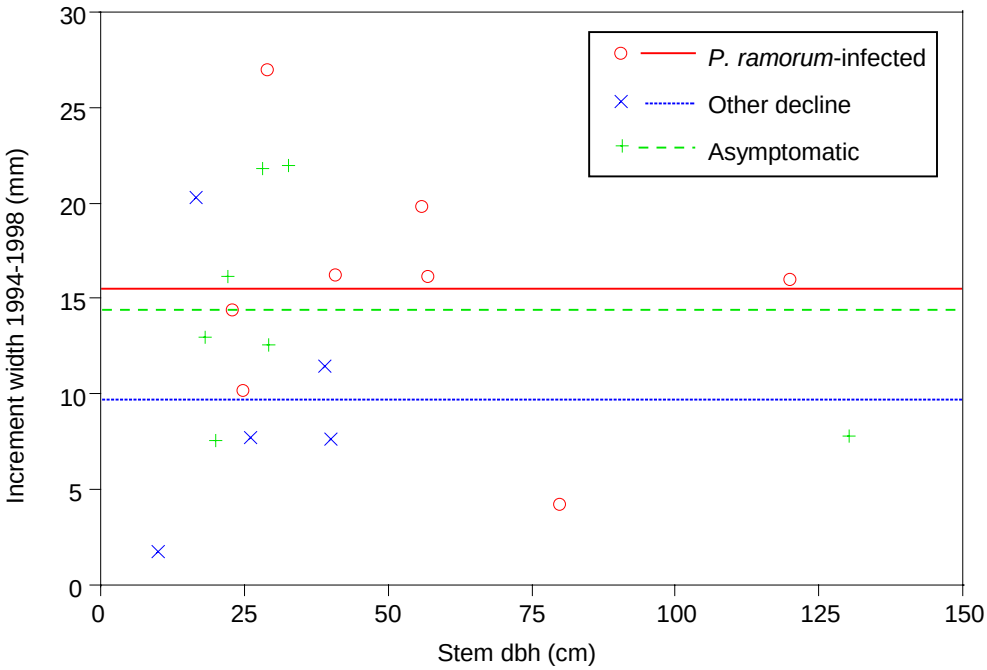

Figure 16. Five year (1994-1998) growth increment width and stem diameter for trees at 5 study locations. Sample includes dead trees within plots with *P. ramorum* canker symptoms and living trees located beyond plots that lacked symptoms of *P. ramorum* infection. The latter group includes both healthy trees (asymptomatic) and trees declining due to causes other than *P. ramorum* canker (other decline). Horizontal lines represent average increment widths for each group. Mean increment widths do not differ significantly between the three groups in this limited sample.

DISCUSSION

Disease levels and disease progress

Data from other studies, including artificial inoculation of both seedlings and mature trees (Rizzo and others 2002a) have indicated that tanoak is more susceptible to *P. ramorum* infection than is coast live oak. Over the past three years, mortality rates due to *P. ramorum* have been greater for tanoak than for coast live oak (Figure 9), which is consistent with the greater disease susceptibility of tanoak seen in other studies. Infection rates for tanoak are also higher than for coast live oak (Figures 7,8), which may reflect the greater susceptibility of tanoak. However, higher levels of infection could also result if tanoaks were exposed to greater levels of inoculum than coast live oaks. *P. ramorum* sporangia are produced on tanoak twig cankers (Dave Rizzo, personal communication), whereas sporangium production has not been observed in the canopy of coast live oak.

Few newly-symptomatic trees have been observed in plots between 2000 and 2002. The rate of new symptom development is also low relative to the overall infection rate (Figures 7,8). Given that disease symptoms have progressed slowly in many of the trees, it is possible that many or all of the newly symptomatic trees were infected prior to 2000 but had not developed external symptoms by that time.

In artificially-inoculated coast live oak, external symptoms of *P. ramorum* canker have developed in some trees within three weeks after inoculation (Rizzo and others 2002a). However, even in inoculated trees, the timing and extent of symptom development has been highly variable

(Rizzo and others 2002a). In addition, in tanoak especially, even trees that are killed by *P. ramorum* cankers sometimes fail to show bleeding or other externally obvious evidence of canker development. This suggests that at least a small percentage of *P. ramorum* cankers can remain externally undetectable for an extended period of time. Such cryptic infections could account for the small number of newly symptomatic trees observed to date.

Even if some or all of the few new infections were initiated after September 2000, it is clear that the rate of disease onset among coast live oak and tanoak populations in the study areas has decreased substantially from the levels experienced at the peak of the current epidemic. Reduced levels of new infections may be due to several factors. Between 1995 and 1998, the study areas experienced three high rainfall years, each of which had relatively high amounts of rainfall in May. Although no systematic surveys were conducted during this period, various observers noted that the appearance of new disease symptoms within the study area was high between about 1997 and 2000 (Svihra 2001). Rainfall amounts and timing may have been less favorable for inoculum production since 1998, due to lower total rainfall amounts (Figure 3) and the lack of substantial late spring rains between 1999 and 2002.

Furthermore, many of the trees in the areas that have the highest risk of infection may have already been infected. Remaining trees may have higher levels of resistance and/or be physically located further from sources of inoculum (e.g., heavily infected California bay trees). The relative contributions of these various factors can be assessed by correlating further observations of disease onset with environmental conditions, measurements of inoculum production, and ratings of tree resistance.

By taking a variety of health ratings on trees each year, we have been able to clearly document that many *P. ramorum*-infected trees exhibit a pattern of disease progress that differs from the "sudden oak death" pattern of disease noted when the disease was initially recognized (Rizzo and others 2002a). Trees exhibiting the acute disease syndrome show a relatively synchronous necrosis of all canopy leaves over a relatively short time period. In contrast, the chronic disease syndrome is characterized by a gradual thinning and dieback of fine branches throughout the canopy of trees with extensive but incomplete girdling of the bole by cankers. In some of these trees, the thinned canopy eventually undergoes a synchronous necrosis similar to that seen in the acute syndrome. Other trees showing the chronic decline syndrome have been killed by bole failures that occurred while the canopy was still green. However, additional years of observations will be needed to determine the eventual disposition of many trees that currently show chronic decline symptoms.

Factors associated with disease occurrence and progress

Factors associated with disease occurrence in logistic regression models have been discussed extensively in two previous annual reports (Swiecki and Bernhardt 2001a, 2002a) and are not discussed again in detail here. Canopy cover of bay within plots was a significant predictor of disease, but was not a better predictor than the total bay density within the plot. Although the presence of poison oak within plots appeared in our models based on 2001 data, the new variable poison oak cover was not significant in the model. Based on the observations from 2002, it does not appear that poison oak is a strong predictor of *P. ramorum* canker occurrence. Given that poison oak is also a host of *P. ramorum*, it is possible that it may be a significant source of inoculum in some situations, such as when trees are covered with the climbing vines that this species forms. Further investigation of the spatial proximity of California bay, poison oak, and possibly other foliar hosts of *P. ramorum* are planned for the 2004 field survey.

Based on various factors that have been associated with disease risk in our models, we have hypothesized that susceptibility to *P. ramorum* canker in *Q. agrifolia* may be related to growth rate (Swiecki and Bernhardt 2002a,b). Disease is less common in trees with low stem water potential, high levels of overtopping, decline associated with other agents, and small stem diameter (Swiecki

and Bernhardt 2002a). All of these factors are associated with slow growth, which could indicate that slow-growing trees may be less susceptible to *P. ramorum* canker. We attempted to study this possibility by directly measuring increment growth in diseased and non-diseased trees.

Results of our pilot study on the relationship between increment growth and disease susceptibility were inconclusive. The methods we used were not optimal for studying this relationship because characteristics of the wood and growth increments made it very difficult to determine the size of the annual increments with a high degree of confidence. Because many of the cores from dead trees were unusable due to decay, we believe that a study of growth rates would have to include a large number of living trees with *P. ramorum* symptoms. In addition, larger-diameter cores or preferably disks should be sampled to obtain more reliable increment measurements. However, these methods are incompatible with monitoring disease progress in undisturbed trees, which is a major focus of our ongoing study.

Data from our pilot study of increment growth also indicates that differences in increment size between different categories of trees within a stand are likely to be relatively small, so large sample sizes would be needed. Given these parameters, it may be difficult to conduct such a study on an intact stand. However, stands being extensively cut for development or other purposes may provide an opportunity to studying the relationship between growth rate and *P. ramorum* susceptibility in detail.

One final finding of our pilot study is that many of the cores we examined showed relatively wide growth increments close to the pith, but most increments produced over the past few decades have been smaller. We have previously noted that many stands in the study areas are quite dense and that high levels of competition for both light and soil moisture are likely to occur within these stands. Many of the stands have clearly regenerated following various amounts of clearing, and it appears that tree growth rates diminished after high levels of canopy closure were attained some decades ago.

Preliminary disease progress models based on infected case live oak trees do not indicate that any of the variables tested to date are especially strong predictors of disease progress. Small diameter trees were less likely to show disease progress, which could be related to growth rate or other factors, such as the thickness of live bark tissue, the biochemical and/or physiological status of the bark, or other factors. We have included measurements of bark thickness in our 2003 field survey in order to obtain some basic data on the characteristics of bark in infected and noninfected trees and to provide additional tree-related predictor variables for modeling of disease onset and progress.

Tree failure

Failures in the two years since monitoring began in September 2000 have occurred primarily among trees which were dead or in late stages of disease at the start of the study. Bole failures were the most common type of failure observed. We observed the same trends in our larger study of tree failure that involved six of the locations used in this study (Swiecki and Bernhardt 2003). Among trees that were dead due to *P. ramorum* in September 2000, large numbers of failures began to occur after September 2001, and more than half of these trees had failed by September 2002. By comparison, the failure rate for trees with late *P. ramorum* canker symptoms in 2000 was 37% after two years, and was only 5% for trees with early *P. ramorum* canker symptoms in 2000.

Given the lack of failures in asymptomatic trees over the same period, these results show that the likelihood of tree failure is greatly increased in trees with late *P. ramorum* canker symptoms and trees that have been killed by *P. ramorum*. This is also consistent with results reported in our other study (Swiecki and Bernhardt 2003). Data collected in this study allow us to track failures over time and determine which factors may be indicators that failure is likely to occur within the following year. However, we have deferred detailed modeling of failure over time until we have accumulated failure data over a longer period.

Water relations

September SWP measurements made on individual trees in 2000, 2001, and 2002 were highly correlated. This shows that September SWP readings provide a consistent measure of the relative degree of water stress experienced by specific trees by the end of the dry season. Furthermore, the correlation between SWPs measured on multiple trees within the same plot indicates that much of the variation in SWP is a response to local soil moisture levels within the plot area. Overall, SWP of the monitored trees seems to be primarily related to seasonal rainfall and evapotranspiration.

Coast live oaks that maintain high SWP levels through the end of the growing season have a greater risk of developing *Phytophthora* canker symptoms than do more water-stressed trees. As noted previously (Swiecki and Bernhardt 2001a, 2001b, 2002a), this finding was the opposite of the effect that we had initially anticipated. In addition, September SWP readings have not declined in *P. ramorum*-infected trees over time. As noted in the results, about a third of the trees with late *P. ramorum* canker symptoms have exhibited higher than expected September SWP readings.

Because many trees with late *P. ramorum* canker symptoms have developed diffuse crown thinning and dieback, their leaf area and presumably total evapotranspiration (ET) is reduced. If water uptake is not impaired and ET is reduced, SWP should increase, provided that water transport through the xylem is adequate. This hypothesis is further supported by our observations that basal sprouts of trees with killed tops maintain high SWP readings. It appears from our data that *P. ramorum* infection does not cause a gradual decrease in SWP over time. Disease-induced decline in SWP appears to be limited to a very late stage of the disease syndrome associated with destruction of the xylem by wood decay fungi.

Trees rated as in severe decline (i.e., likely to die in 10 years) had the highest water potentials overall. These trees are often highly suppressed understory trees with low canopy exposure to light. Relative to other symptom classes , the decline class has the highest overall levels of canopy dieback and thinning. These trees presumably have low evapotranspiration demand, and similar to the situation seen above, tend to have relatively high SWP readings indicating low water stress.

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