Factors related to *Phytophthora* canker (sudden oak death) disease risk and disease progress in coast live oak and tanoak

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Cover photos:
Left - First author (Swiecki) measuring stem water potential (SWP) using pump-up pressure chamber.
Right - Symptomatic (case) subject coast live oak with evident bleeding cankers. Leaves for SWP measurements are enclosed in reflective bags.

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SUMMARY

This report presents data from the second year of observations in a case-control study to examine the role of water stress and various other factors on the development of *Phytophthora* stem canker disease (commonly called sudden oak death) in coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*). The study compares subject trees that exhibited symptoms of *Phytophthora* infection (case trees) with symptomless (control) trees. In September 2000 and September 2001, we collected data in 150 circular plots (8 m radius) in areas where disease caused by *Phytophthora 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.

Various plot and tree factors were associated with disease in the subject tree in logistic regression models for coast live oak. Vegetation-related plot variables that were positively correlated with disease in coast live oak 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-related factors that were associated with disease included multiple stems, large stem cross-sectional area, high levels of canopy exposure, and high stem water potential (SWP). In addition to these factors, logistic 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*.

The direction of the effects of a number of variables in several different analyses suggests the possibility that *Phytophthora* canker in coast live oak is more likely to occur in trees that are vigorous and/or fast-growing (larger, more dominant, less water-stressed, not in decline due to other agents) than in trees that are suppressed and/or slow-growing. Significant positive correlations between canopy dieback and *Phytophthora* canker may indicate that diffuse dieback in the canopy is an early indicator of *Phytophthora* canker for both coast live oak and tanoak.

Disease progress in trees with symptoms of *Phytophthora* canker was more rapid for tanoak than for coast live oak. *Phytophthora*-related mortality between 2000 and 2001 was greater for tanoak (10% of cases, 19% of symptomatic plot trees) than for coast live oak (3.8% of cases, 6% of symptomatic plot trees). New *Phytophthora* canker symptoms were also more common in tanoak than in coast live oak (8% and 1.6% of previously asymptomatic plot trees, respectively). Somewhat less than half of the coast live oak and tanoak cases showed no obvious advancement of disease symptoms between 2000 and 2001. Preliminary disease progress models for coast live oak indicate that most of the factors associated with disease occurrence are not associated with disease progress in trees that are already symptomatic. This pattern would be consistent with a disease model in which infection events occur infrequently and disease progress is due primarily to canker expansion rather than the initiation of additional cankers.

Subject tree SWP readings for 2001 were 0.54 MPa lower on average than 2000 SWP readings across both species. SWP readings for individual trees in both years were highly correlated. SWP readings made on multiple trees within plots were correlated, suggesting that plot soil moisture levels account for much of the variation in SWP between plots. Trees with *Phytophthora* canker symptoms did not show a significant overall reduction in SWP between 2000 and 2001 relative to asymptomatic trees, and trees that died between 2000 and 2001 had higher than average SWP readings in 2000. Disease progress was not correlated with changes in SWP. These results and other observations indicate that canopy water stress associated with the final drying of the top occurs over a period of less than a year.

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*). The disease has been referred to as "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 study has 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*, oak bark beetles (*Pseudopityophthorus* spp.), and ambrosia beetles (*Monarthrum* 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).

At the time this study was initiated in August 2000, very little was known about the epidemiology of this disease. Water stress had been considered as 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. Descriptive case-control studies are designed to examine how past (retrospective) factors are related to the current health of individuals. Such studies are commonly used in medical research to examine connections between risk factors and diseases (e.g., history of smoking and lung cancer).

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 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. In addition, by reassessing disease observations on an annual basis, we can begin to assess risk factors associated with disease progress and eventual mortality. This paper reports on the second year of observations for this ongoing study. Results from the first year's data (2000) have been previously reported (Swiecki and Bernhardt 2001a, 2002)

Although *P. ramorum* is the most common pathogen associated with bleeding aerial bark cankers in stands affected by SOD, at least one other previously unrecognized *Phytophthora* species has also been

associated with bark cankers in tanoak and coast live oak (Rizzo and others 2002b). This *Phytophthora* is not yet completely identified and is currently referred to as a "*P. ilicis*-like" species. At this time, it is not possible to clearly differentiate between stem cankers caused by *P. ramorum* and those caused by the *P. ilicis*-like species on the basis of field symptoms alone. Although plots were established in areas in which the presence of *P. ramorum* has been confirmed, in this report we use the general term *Phytophthora* canker to account for the possibility that some of the observed aerial bark cankers could be caused by *Phytophthora* species other than *P. ramorum*. If the *P. ilicis*-like species or other *Phytophthora* species are the cause of some of the observed bark cankers, our observations in some locations would apply to a *Phytophthora* disease complex rather than to disease caused by *P. ramorum* only. While this complicates interpretation of results by adding to the number of possible explanations for observed relationships, the design of the study is valid for either a single disease or a disease complex.

METHODS

Study site selection

During September 2000, we established plots at 12 study locations (Table 1). Study sites were selected on the basis of appropriate vegetation type (adequate representation of coast live oak or tanoak), the presence of cases and controls 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. This minimizes the likelihood that controls were simply trees that had not been exposed to *Phytophthora* inoculum, although this possibility cannot be ruled out for all controls. 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

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 around 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. We recorded distance and azimuth readings between plots using a survey laser to identify the locations of subject trees within each study site. 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 due to poor satellite reception.

Stem water potential measurements

In September 2000 and September 2001, we collected midday stem water potential (SWP) readings on the center subject tree in each plot during the peak midday period (about 1-3 pm PDT) following methods outlined by Shackel (2000). On each tree, we selected a minimum of two leaves or shoot tips with several leaves that branched directly off the trunk, from main branches near the trunk, or from basal sprouts (primarily for tanoak). Each leaf or shoot tip was sealed in a clear plastic bag and overbagged with a larger opaque reflective plastic bag. These bags prevent transpiration and excessive heating of the leaf. 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 leaf or shoot tip was excised and placed into the pressure chamber while still sealed in the inner plastic bag. Generally only one shoot tip was needed to determine SWP, but two readings were made on many trees as a check on the technique. 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). In both 2000 and 2001, 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, 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.

Additional tree and plot variables

The change in *Phytophthora* canker disease status between September 2000 and September 2001 was determined for each subject tree and for all coast live oak, black oak, and tanoak trees in each plot. Other plot and tree variables we assessed are listed in Tables 2 and 3. Basal area was measured by the Bitterlich method using a variable radius plot in 2000 only. The remaining plot-related variables were assessed on an 8 m radius fixed-area plot centered at the subject tree. The measured variables were also used to calculate a number of additional variables for various analyses. 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 statistical software (SAS Inc., Cary NC) for data analysis. Unless otherwise indicated, effects or differences are referred to as significant if $P \leq 0.05$.

Logistic regression models

We used single and multiple variable logistic regression models to screen over 65 tree- and plot-related predictor variables against two binary outcome variables for coast live oak and/or tanoak. For clarity, both predictor and outcome variable names are shown in uppercase letters in the text. The outcome variables of interest were:

CASE 2001 - subject trees classified as showing *Phytophthora* canker symptoms in 2001

DISEASE PROGRESS - a subset of CASE 2001 including only those trees showing a progression in *Phytophthora* canker symptoms between September 2000 and September 2001.

For coast live oak, DISEASE PROGRESS models were developed using only cases, so that only factors affecting disease progress but not initial disease onset were under study. For tanoak, the sample size was insufficient to develop models for the DISEASE PROGRESS outcome.

Possible predictor variables were only considered if there was enough variation in the levels of the variable to be meaningful. For example, some plot tree and shrub species occurred so rarely in plots that the presence of these species in plots is not a meaningful variable. We also looked for correlations between predictor variables and checked selected predictor variable distributions to determine whether the models were overly influenced by a few outlying observations. Factors were generally considered for entry into the multivariate models if odds ratios from univariate models were significant at $p \leq 0.10$, but factors that were not significant in univariate models were also tested in multivariate models.

The likelihood ratio chi square is used to test the significance of each effect in the model. Likelihood ratio chi square tests are calculated as twice the difference of the log likelihoods between the full model and the model constrained by the hypothesis to be tested, i.e., the model without the effect (SAS Institute 2000). The reported significance level of each factor in a multivariate model is therefore dependent upon the other factors which are included in the model. Hence, the significance level of each factor reported in the models should be interpreted as if it were the last factor added to the model. We also calculated Akaike's information criterion (AIC) to compare the fit of alternative models. For models constructed for a given data set, smaller AIC values indicate better model fit.

The parameters obtained from nominal logistic regression models are odds ratios (Breslow and Day 1980). The odds ratio is the odds of an outcome given exposure to a factor divided by the odds of an outcome given no exposure to the factor, i.e.:

$$\text{odds ratio} = \frac{\text{odds}(\text{case} \mid \text{exposure})}{\text{odds}(\text{case} \mid \text{no exposure})} \qquad \text{(Equation 2)}$$

The odds that a tree will be diseased (a case) given exposure to a factor *x* is the probability of disease given exposure to *x* divided by the probability of no disease (i.e., tree is a control) given exposure to *x*. Mathematically this is expressed as follows:

$$\text{odds}(\text{case} \mid x) = \frac{\text{P}(\text{case} \mid x)}{1 - \text{P}(\text{case} \mid x)} = \frac{\text{P}(\text{case} \mid x)}{\text{P}(\text{control} \mid x)} \qquad \text{(Equation 3)}$$

An odds ratio of one indicates that the factor has no effect on the outcome variable. If the odds ratio is greater than one, an outcome is more likely if a tree is exposed to factor *x*. If the odds ratio is less than one, an outcome is less likely if a tree is exposed to factor *x*. For binary predictor variables (i.e., those that have only two levels), the odds ratio indicates how many times more likely the outcome is when exposure to the factor is present compared to when it is absent. An odds ratio much higher than one indicates that a factor has a strong positive effect on an outcome. An odds ratio much smaller than one (i.e., the reciprocal is a large number) indicates that a factor has a strong negative effect on the outcome.

For continuous variables (i.e., variables that vary more or less continuously), the odds ratio is based on each increment of the variable, e.g., per MPa difference in SWP. For this example, the odds ratio for *n* MPa difference in SWP would be the per MPa odds ratio raised to the power *n*.

Other linear models and tests

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 Fisher's exact test on 2 × 2 contingency tables to test for the significance of differences between two proportions. Significance levels for Fisher's exact test are for two-tailed probabilities unless otherwise noted.

Table 2. Tree variables measured for subject trees (and additional trees used for water potential measurements) in each study plot

Variable	Method	Scale/units and notes
Tally tree species		*Q. agrifolia* or *L. densiflorus*
Origin class	visual assessment	seed (0) or sprout (1)
Distance to plot center	laser rangefinder	m; recorded for additional water potential trees
Azimuth to plot center	compass	degrees; recorded for additional water potential trees
DBH	flat tape	cm
Number of stems from ground	count	stems/tree
Stems with *Phytophthora* symptoms	count	infected stems/tree
Dead stems	count	dead stems/tree
Tree dead / cause	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	presence of fruiting bodies	present (1) / absent (0)
Bark and/or ambrosia beetles in main stem	presence of boring dust and/or holes	present (1) / absent (0)
Phytophthora-related symptoms	visually assess symptoms present	(0) No symptoms (1) Early - bleeding cankers only (2) Late - cankers plus beetles and/or *H. thouarsianum* (3) Dead as result of *Phytophthora* infection; evidence of bark cankers present
Recent bleeding from cankers	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	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	visual estimate	pretransformed 0-6 scale[1] 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. In 2001, individual ratings were also made for each stem of multistemmed trees.
Severe tree decline due to other agents	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.
Sky-exposed canopy	visual	pretransformed 0-6 scale[1]; percent of canopy projection area with unobstructed access to direct overhead sunlight
Canopy thinning	visual estimate	0-2 Scale: 0-none, 1-slight, 2-pronounced
Canopy dieback	visual estimate	pretransformed 0-6 scale[1] Based on percent dead crown volume

Decay impact	visual assessment	0-3 Scale: 0-no , 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 (0); 2=low(1) or moderate (2); 3=high (3)
Status change	comparison of 2000 and 2001 data	(0) no change; (1) improved condition; (-1) degraded condition
Epicormics	visual assessment	0-2 Scale: 0-none, 1-few, 2-numerous
Live basal sprouts	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.

[1]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. All variables were measured in the 8 m radius fixed-area plots.

Variable	Method	Scale/units and notes
Tree density / species composition	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	clinometer	percent
Plot aspect	compass	degrees
Plot drainage	visual observation	none (N); creek/drainage with surface water (WC); dry creek or drainage (DD)
Plot drainage proximity	visual observation	0 if in plot; otherwise estimate meters from plot edge
Plot tree canopy cover	visual estimate	pretransformed 0-6 scale[1]
Plot shrub cover	visual estimate	pretransformed 0-6 scale[1]
Overstory canopy trees species in plot	visual assessment	list of species Overstory canopy trees do not have to be rooted within the plot.
Count by tree health class relative to *Phytophthora* canker symptoms and other decline/mortality agents (SOD hosts[2] only)	tree count by species, subcategorized by symptom class and canopy position (overstory/understory where overstory trees have sky-exposed canopy rating 2 or higher)	Symptom classes are based on combinations of tree death causes, *Phytophthora* symptom classes, and severe decline ratings (Table 2): 0 - asymptomatic 01 - early *Phytophthora* canker disease 02 - late *Phytophthora* canker disease 03 - dead attributed to *Phytophthora* canker 10 - severe decline due to other agents 20 - dead due to other agents 30 - dead but cause can't be determined 11 - early *Phytophthora* disease **and** severe decline due to other agents 12 - late *Phytophthora* disease **and** severe decline due to other agents 23 - dead attributed to both *Phytophthora* and other agents -Other decline/mortality agents do not include *H. thouarsianum* and bark or ambrosia beetles if they are associated with *Phytophthora*
Count by general tree health class (trees other than SOD hosts[2])	tree count by species, subcategorized by symptom class and canopy position (overstory/understory)	Symptom classes: 0 - live 10 - decline 20 - dead
SOD host[2] regeneration	count or estimate if >10	regeneration = seedlings and saplings <3 cm dbh
Disease incidence in SOD host[2] regeneration	count or estimate percent if count > 10	Disease may be due to *P. ramorum* and/or other agents or factors
Dead SOD host[2] regeneration	count	Cause of mortality in regeneration was not determined
Regeneration of trees other than SOD hosts[2]	presence noted by species	regeneration: seedlings and saplings <3 cm dbh
Other pathogens/agents	note presence	listing of agents and symptoms observed, including various decay fungi, canker rot, root disease, *H. thouarsianum*, and beetles
Woody understory species	note presence	list shrubs and woody vines present within plot; herbaceous species and grasses were not scored
Disturbance	Note type of disturbance	roads, trails, logging, etc. within plot or near edge of plot were noted
Oak/tanoak failure in plot	count	Bole and major limb failures observed in the plot were noted.

[1]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%

[2]Hosts of *Phytophthora* canker, i.e., coast live oak, black oak, and tanoak

RESULTS

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

Comparison of 2000 and 2001 stem water potentials

Overall, 2001 stem water potential (SWP) readings were significantly lower than 2000 readings (Figure 1), averaging 0.54 MPa less in 2001 than in 2000 (paired t-test $p<0.0001$). We used regression analysis to determine whether the difference between 2000 and 2001 SWP readings varied by species or vapor pressure deficit (VPD) difference between the two years. Neither factor was significant at $p\leq0.05$, although the VPD difference (2000 VPD-2001 VPD) was significant at $p=0.0588$.

The effect of study location on SWP difference was tested in separate analyses for each species. The difference between 2000 and 2001 SWP readings varied significantly between locations (one-way analysis of variance F test $p=0.0379$ and 0.0177 for coast live oak and tanoak, respectively).

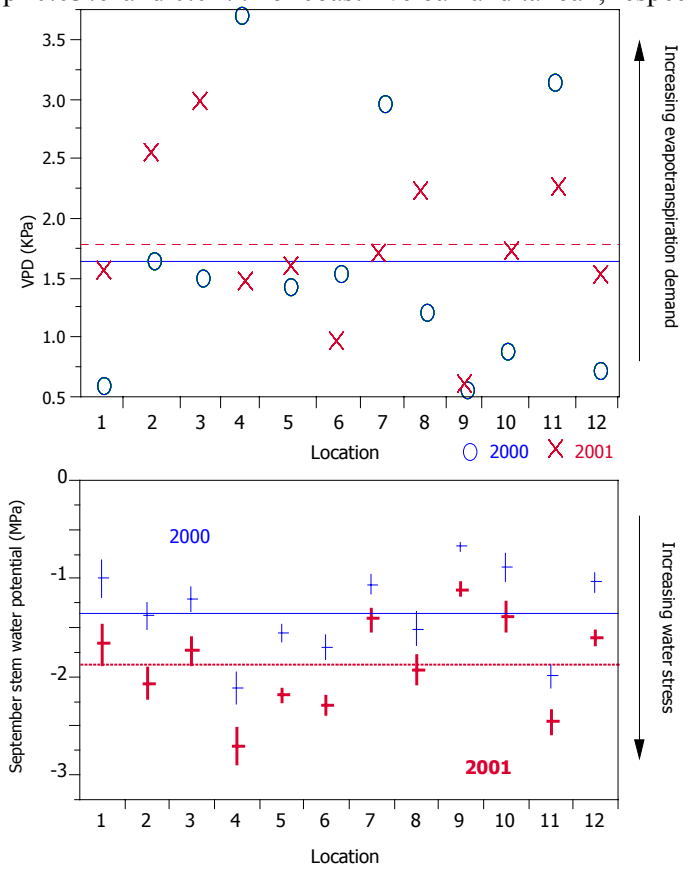

Figure 1. Top graph: Calculated average vapor pressure deficits (VPD) for each location during the period that stem water potentials were measured. (blue O=2000 data, red X=2001 data). The overall average for each year is shown by a horizontal line (solid blue line=2000 average, dashed red line=2001 average).
Bottom graph: Average stem water potentials for trees at each location in 2000 (thin blue dashes) and 2001 (bold red dashes). Vertical lines represent standard error of the mean and the overall mean for each year is shown by a horizontal line (solid blue line=2000, dashed red line=2001). At locations 9 and 12, subject trees are tanoak; at all other locations subject trees are coast live oak.

From the consistently lower SWP values obtained at all locations and for both species in 2001, we infer that soil moisture levels in September 2001 were generally lower across the region than they were in September 2000. This is consistent with rainfall records for the rainfall seasons (July-June) preceding each set of readings. For San Rafael, which is located near most of the Marin County sites, 1999-2000 rainfall was 69.0 cm compared with 50.7 cm in 2000-2001. The 30-year average rainfall (1971-2000) for San Rafael is 84.8 cm. Other weather stations in the area show similar rainfall trends for these two years.

SWP measurements made in 2001 were highly correlated with readings on the same trees made in 2000 (adjusted R^2= 0.784, Figure 2). In general, most trees retained their SWP ranking relative to other trees; e.g., trees with higher than average SWP in 2000 generally had higher than average SWP in 2001. Hence, September SWP readings appear to be a reasonably repeatable indicator of tree water stress and site characteristics that are related to soil water availability.

With one exception, 2001 SWP readings for subject trees were equal to or less than 2000 SWP readings. The maximum observed difference between 2000 and 2001 SWP values was 1.5 MPa. The one tree that had a substantially higher SWP reading in 2001 (-1.15 MPa) than in 2000 (-1.425 MPa) was adjacent to a small stream, the flow of which is controlled artificially. This stream was dry in 2000 but had running water at the time of measurement in 2001. It appears that the stream had artificially elevated the soil moisture within this tree's rootzone in 2001, resulting in its aberrantly elevated SWP.

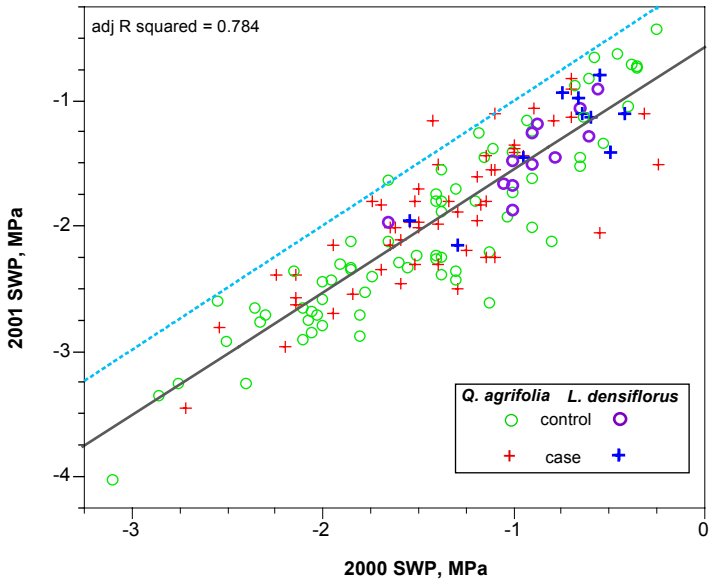

Figure 2. Correlation between 2000 and 2001 stem water potential (SWP) readings. Solid line represents the regression line. Dashed line represents the line of equal readings for both years (slope=1 and intercept=0).

In 2000 we measured SWP of only the center subject tree (case or control) in each plot. To gather information on how representative these SWP values were of the plot water status as a whole, in 2001 we gathered SWP readings from additional plot trees in 45 of the 150 plots (one additional tree per plot except one plot with two additional trees). Readings between pairs of trees from the same plot are significantly correlated (Figure 3). This suggests that much of the variation in SWP is related to the available soil moisture level within the overall plot area, which is a function of soil type and depth, slope, aspect, and vegetative cover. Therefore, it appears that SWP readings of either the subject tree or another tree within the plot are indicative 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 water stress as a factor for plots in which the subject tree has died.

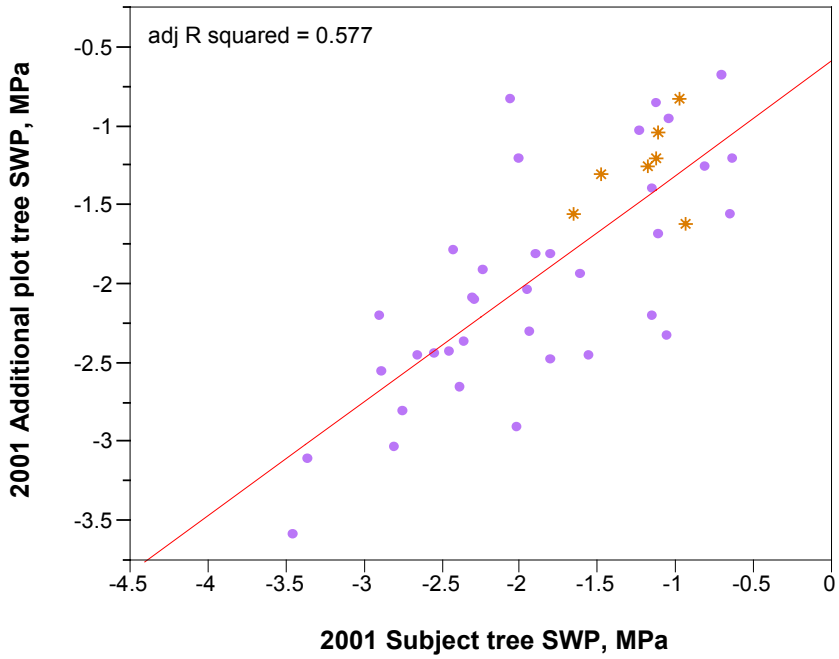

Figure 3. Correlation between stem water potential (SWP) of subject trees and SWP of an additional tree within the same plot. Purple dots = coast live oak, red asterisks = tanoak. The regression line is the solid red line.

Factors associated with SWP

We used regression analysis to evaluate whether tree and plot factors were related to the variation in SWP. In both 2000 and 2001, although the range of SWP values recorded for tanoak fell completely within the range of coast live oak SWP values (Figure 1), average SWP for tanoak was significantly higher than that of coast live oak (t-test $p<0.0001$ for both years). Average SWP readings for tanoak and coast live oak differed by 0.71 MPa in 2000 and 0.76 MPa in 2001. 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.

Coast live oak

Regression models for 2001 SWP readings accounted for about one-third of the variation in SWP (Table 4). As observed in analyses of 2000 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 low SWP values (i.e., elevated levels of water stress).

Several other variables related to *Phytophthora* canker symptoms could be substituted into the regression model in place of *PHYTOPHTHORA* GIRDLING RANK without substantially altering model fit. These include *PHYTOPHTHORA* CLASS (asymptomatic, early, late), *PHYTOPHTHORA* CANKER COUNT, and CASE 2001 (i.e., the binary disease presence variable). For all of these disease variables, *Phytophthora* symptoms were associated with higher SWP readings, i.e., disease was more common in trees that exhibited relatively low levels of water stress.

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 coast live oak SWP readings. 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. This situation may exist in most of our coast live oak study areas. Seven coast live oaks at location 3 that had an overall average SWP of -1.45 MPa on 8/17/2000 also had stomatal conductance readings that averaged a relatively low 84.5 mmol/s/m^2. Given that the overall average SWP for all coast oaks in September 2001 was -1.86 MPa, we would expect more stomatal closure and less of a relationship between SWP and VPD in 2001 than in 2000.

In September 2001 we used two probes to record temperature and relative humidity data simultaneously at the top of the canopy and about 2 m above ground level during the period when SWP measurements were made. Only a single probe set near the top of the canopy was used in 2000. Canopy and near-ground VPD measurements were highly correlated (adjusted R^2=0.955) and the difference between paired ground and canopy VPD measurements was not significant (paired t-test). These data indicate that no substantial VPD gradients existed between the top of canopy and the ground at the time that SWP measurements were made. This suggests that the lack of correlation between VPD and SWP among trees with low canopy exposure in 2000 and all trees in 2001 was more likely related to stomatal closure than to differences in VPD at different heights within the canopy.

Table 4. Regression model for midday stem water potential (MPa) of coast live oak in September 2001

Source	DF	F Ratio	Prob>F	Adjusted R^2	n
Overall model	3	29.5492	<0.0001	0.3431	165
Model terms	**DF**	**F Ratio**	**Prob>F**	**Parameter estimate**	
Sky-exposed canopy rating	1	62.777	<0.0001	-0.2092	
Plot annual insolation (MJ/m^2)	1	5.265	0.0231	-0.000121	
Phytophthora girdling rank	1	15.649	0.0001	0.1151	
Intercept				-0.5187	

SWP and disease progress. For coast live oak cases, neither 2001 SWP nor the change in SWP readings from 2000 to 2001 were significantly correlated with disease progress. SWP readings for 2001 did not differ significantly between trees with early *Phytophthora* symptoms (bleeding cankers only) and late symptoms (cankers plus beetles and/or *H. thouarsianum* sporulation). Furthermore, disease progress (expansion of existing cankers and/or development of late disease symptoms) was not correlated with any of the SWP variables.

Despite the lack of an overall relationship between SWP and disease progress, we were able to observe the effects of the terminal phase of disease on SWP in one tree at location 10 (Napa County). This tree had extensive canker development but was well foliated in 2000. By September 2001, the trunk showed abundant sporulation of *H. thouarsianum* and only a few live leaves were left on the main stem, at a height of about 6 m. In September 2000, this tree had a higher than average SWP of -0.55 MPa, but the September 2001 SWP was -2.05 MPa. The substantial drop in SWP observed in this nearly dead tree is consistent with a drying out of the top as the result of sapwood destruction by *H. thouarsianum* and/or destruction of the root system by *Armillaria mellea*.

Two other coast live oak subject trees were completely dead by September 2001, so SWP could not be determined for those trees. In September 2000, neither of these trees showed evidence of water stress, and had SWP readings of -0.9 and -1.15 MPa, higher than the overall September 2000 average for coast live oak (-1.34 MPa). Hence, these trees transitioned from having green canopies with low levels of water stress to complete canopy death in less than a year.

The above observations suggest that coast live oaks affected by *Phytophthora* canker do not undergo a gradual decline in SWP as disease progresses over a period of years. Rather, it appears that the decline in SWP may occur over a fairly short time frame, something less than one year and perhaps as little as a

few months or weeks before the tree is completely dead. Additional SWP observations in 2002 should provide a more detailed picture of changes in SWP that may precede tree death.

Tanoak

The regression model for tanoak SWP using both subject trees (n=22) and additional plot trees with SWP measurements (n=7) is shown in Table 5 below. The most significant predictor in this model is location. Because tanoak observations were limited to two locations, location is completely confounded with other location-specific variables such as VPD. If VPD is used in the model rather than location, the effect is in the expected direction, i.e., SWP was low when VPD was high. However, we cannot be certain whether the significance of the location effect is due to VPD or other location-specific factors such as seasonal evapotranspiration, rainfall, and soils.

Plot annual insolation was significant in the tanoak SWP regression model (p=0.0007), as it was in the coast live oak model. The direction of this effect is as expected, i.e., SWP decreases as insolation (and therefore evaporative demand) increases.

Variables related to *Phytophthora* canker symptoms were not strongly correlated with SWP in tanoak. The binary variable DISEASE PROGRESS, which denotes cases in which disease progress had occurred between 2000 and 2001, was significant at $p \leq 0.10$ when included as a variable with the other variables shown in Table 5 (overall model $p<0.0001$, adjusted $R^2=0.655$, n=22, DISEASE PROGRESS $p=0.0551$, LOCATION $p<0.0001$, PLOT ANNUAL INSOLATION $p=0.0322$). This model indicates that SWP was higher in case trees that showed visible disease progress in the preceding year, i.e., disease progress was associated with lower levels of water stress. Additional (non-subject) trees are not included in this model because detailed disease progress data (2000-2001) was not available for them.

SWP readings on most tanoaks were taken on basal sprouts because these were often the only leaves within reach. Tanoak SWP measurements in 2001 included data from basal sprouts of two trees with *H. thouarsianum* sporulation, one of which had a dead top. The SWP readings for these trees were -0.795 and -0.925 MPa, which rank in the highest quartile of all tanoak SWP readings. These readings are consistent with expectations if we assume that decline and death of the top has preceded the decline of the root system in these trees. Partial or complete loss of the top greatly reduces plant water use and leads to a high root:shoot ratio, so the water supply of these basal sprouts should be high relative to transpiration demand, leading to elevated SWP values in these trees.

Table 5. Regression model for midday stem water potential (MPa) of tanoak in September 2001

Source	DF	F Ratio	Prob>F	Adjusted R^2	n
Overall model	2	20.818	<0.0001	0.5860	29
Model terms	**DF**	**F Ratio**	**Prob>F**	**Parameter estimate**	
Location [or Vapor pressure deficit (KPa) [1]]	1	41.519	<0.0001	0.298	
Plot annual insolation (MJ/m^2)	1	7.829	0.0096	-0.000044	
Intercept				-0.639	

If VPD is used in place of Location, parameter estimate is -0.647 and intercept is 0.0204.

Disease and disease progress in subject 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, we were able to determine how symptoms progressed during the intervening year and how many previously asymptomatic trees developed new *Phytophthora* canker symptoms. Subject trees include 128 coast live oaks and 22 tanoaks.

Changes in disease status of coast live oak subject trees

Changes in condition and disease symptoms of coast live oak subject trees are diagrammed in Figure 4 and are summarized in Figure 5. Three subject trees scored as controls in 2000 had evidence of *Phytophthora* cankers in 2001 (Figures 4, 5) and have therefore been reclassified as cases in 2001, increasing the total number of cases from 53 in 2000 to 56 in 2001. Disease symptoms intensified in 59% of the 53 original coast live oak subject trees defined as cases in 2000. Among all trees that exhibited obvious disease progress (including the new cases), most (22/34) showed evidence of recent bleeding (within about the past 4-6 months) from cankers. In contrast, only 3 of the 22 cases that showed no change in disease severity were scored as having recent bleeding. Based on increased severity of dieback, thinning, and/or decay impact ratings, 12.5% of the control trees showed a decline in overall condition in the absence of *Phytophthora* canker symptoms (Figure 4).

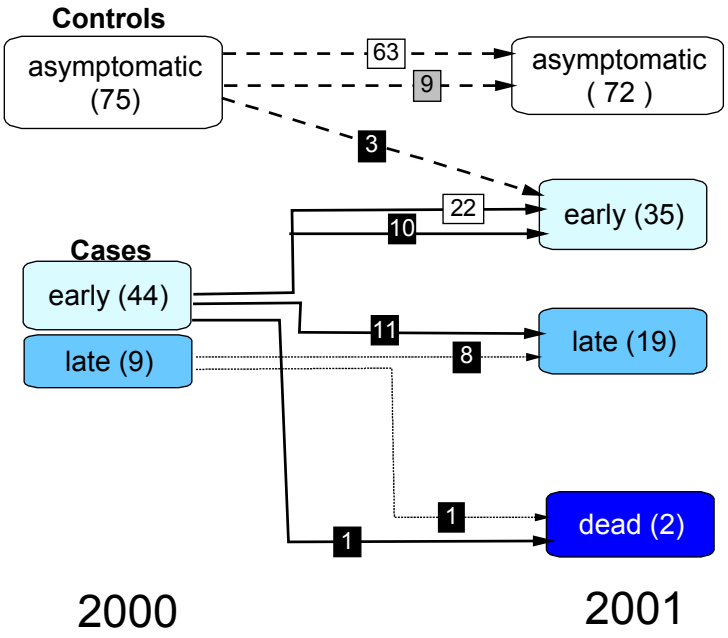

Figure 4. Diagram of transitions related to disease progress in coast live oak subject trees from September 2000 to September 2001. Numbers represent counts of subject trees. White rectangles along arrows represent trees with no apparent change in condition. Black rectangles along arrows represent trees showing increased severity of *Phytophthora*-related symptoms. The gray rectangle along one arrow represents trees showing a decline in health in the absence of *Phytophthora* symptoms.

When this experiment was initiated in September 2000, we had planned to select cases that only had early symptoms of SOD, i.e., bleeding *Phytophthora* cankers only. Late symptoms of SOD-related decline as used herein include bark and/or ambrosia beetle damage and sporulation of the sapwood-decaying fungus *H. thouarsianum*. Because it was difficult to find adequate numbers of trees displaying only early symptoms, 9 of the original 53 coast live oak cases had some limited late disease symptoms, including minor amounts of beetle damage or a few small *H. thouarsianum* fruiting bodies on a single scaffold or on a localized portion of the bole. Trees with these late disease symptoms in 2000 had been noted so they could be handled separately in data analyses. Among the 9 case trees that had late disease symptoms in 2000, one was dead and all exhibited increased disease severity in September 2001. In contrast, 1 of the 44 original case trees with only early symptoms died between 2000 and 2001, and only half (22) exhibited an increase in disease severity.

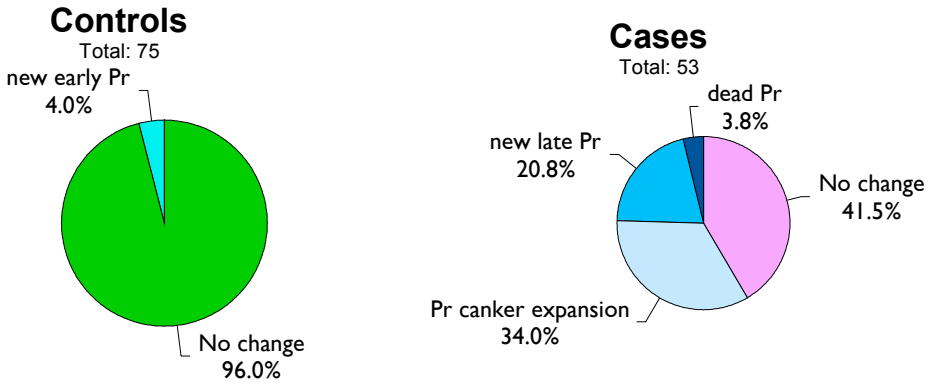

Figure 5. *Phytophthora* canker disease progress in coast live oak subject trees between September 2000 and September 2001.

Factors associated with disease and disease progress in coast live oak

CASE 2001 outcome

Univariate models. Univariate logistic models were primarily used to screen predictor variables to determine which might warrant further analysis and to determine the overall effect direction of the these predictors. Tree and plot factors that were significant predictors of the CASE 2001 outcome in single variable models are listed in Table 6 below. Most of the variables that were significant in the original analyses of the CASE 2000 outcome (Swiecki and Bernhardt 2001a) were also significant predictors of CASE 2001. However, several additional variables that were not previously tested were also significant predictors of the CASE 2001 outcome.

Detailed data on understory shrubs, defined as woody or suffrescent perennial dicots (including vines), were first collected in the 2001 survey. We did not rate cover for individual shrub species because shrub cover was low in most plots. Only 11% of all coast live oak plots had more than 20% shrub cover and 12% had no shrubs present. Shrub diversity was typically low in plots. The median number of shrub species per plot was two, and the maximum observed was six (in three plots). Among the single shrub species variables and summary variables we tested, the only significant predictor of CASE 2001 was the presence of poison oak (*Toxicodendron diversilobum*) in the plot. This variable was significant in both univariate models and multivariate models described below and was positively associated with the CASE 2001 outcome. Composite variables combining poison oak occurrence data with the occurrence of other known shrub hosts of *P. ramorum* (e.g., *Vaccinium, Lonicera, Arctostaphylos*) were not significant. However, a composite binary variable that identifies plots with poison oak and a relatively high overall shrub cover (more than 2.5%) was significant (likelihood ratio p=0.0041). This could indicate that disease risk increases with increasing poison oak cover, but specific poison oak cover data are needed to confirm this possibility.

To interpret the effect direction for the SWP variables it is necessary to bear in mind the fact that more negative SWP values reflect higher levels of water stress. The case outcome is associated with high (less negative) SWP values, so the overall effect direction is positive. The $SWP_{plot\ average}$ variable is the average of the SWP of the subject tree and additional plot tree(s), if any. The $SWP_{plot\ average}$ - $SWP_{location\ max}$ variable compares the plot average SWP to the SWP of the least water-stressed tree at a location. Its value is always negative or zero. The positive effect direction for this variable also indicates that the coast live oaks at each location that show the lowest levels of water stress have the highest risk of disease.

Table 6. Significant predictors of *Phytophthora* infection in subject trees (binary outcome) in univariate logistic regression models for coast live oak.

Predictor variables	Effect direction[A]	CASE 2000 outcome Likelihood ratio Prob > χ^2	CASE 2001 outcome Likelihood ratio Prob > χ^2	2001 DISEASE PROGRESS outcome (for cases only) Likelihood ratio Prob > χ^2
Subject tree variables				
2001 SWP_{tree} - $SWP_{location\ max}$ (MPa)	+		0.0164	ns
2001 $SWP_{plot\ average}$[B] - $SWP_{location\ max}$ (MPa)	+		0.0231	ns
2000 SWP_{tree} - $SWP_{location\ max}$ (MPa)	+	0.0533	0.0708	ns
2001 canopy dieback rating[C]	+		0.0402	ns
2001 canopy dieback >20%	+		0.0112	ns
Decay impact high (rating=3)	+		ns	0.0343
Sky-exposed canopy rating	+	0.0012	0.0056	ns
Sky-exposed canopy >50%	+	0.0003	0.0177	ns
Sum of DBH (cm)	+	0.018[D]	0.0252[D]	0.0238[D]
Sum of stem cross-sectional area (cm^2)	+	0.0461	0.0477	0.0288
Number of stems	+	0.0061	0.0054	ns
More than 1 stem present	+		0.0311	ns
More than 2 stems present	+	0.0007	0.0013	ns
Sprout origin tree	+		0.0267	ns
Plot variables				
Plot canopy cover	-	ns	0.0731	ns
Poison oak present	+		0.0324	ns
Poison oak present & shrub cover > 2.5%	+		0.0041	ns
Live California bay count	+	0.0024	0.0008	ns
Phytophthora canker symptoms present in plot[E]	+	0.0127	0.0010	ns
Count of all trees with *Phytophthora* canker symptoms[E]	+	0.0254	0.0003	ns
Count of trees with only early *Phytophthora* symptoms[E]	+	0.0145	0.0018	ns
Count of trees with early or late *Phytophthora* symptoms[E]	+		0.0010	ns
H. thouarsianum fruiting present	+	0.0021	0.0010	<0.0001
Beetle damage present	+	0.0002	0.0001	<0.0001
Count of live overstory trees other than bay	-		ns	0.0038
All canker hosts (count)	-	ns	ns	0.0417
Madrone overstory present	-		ns	0.0479
Madrone regeneration present	-	ns	ns	0.0121
Coast live oak regeneration count	+	ns	ns	0.0392
> 15 coast live oak seedlings present	+		ns	0.0393

ns = not significant at p<0.10

[A] Effect direction indicates whether outcome is more likely (+) or less likely (-) in the presence of the factor (binary variables) or as the level of the factor increases (continuous variables).

[B] Based on average SWP of subject tree and additional plot tree, if any.

[C] Recoded to 3 classes (2 or less, 3, 4 or more) due to low sample sizes in the lowest and highest classes.

[D] Significance level drops below p=0.05 if 2 high extreme values are excluded.

[E] Predictor variables exclude infection of the subject tree.

Multivariate models. We constructed a wide variety of multivariate models using various combinations of predictors, including both those that were or were not significant in univariate models. Several of the best fitting logistic regression models for the CASE 2001 outcome are shown in Tables 7

and 8. Models using either tree variables or plot variables only were highly significant, but the best models combining both tree and plot variables showed better overall fit than models that used only one set of variables (Table 7). Combined model 1 includes the variable PLOT TREES WITH EARLY *PHYTOPHTHORA* SYMPTOMS, whereas this variable was specifically excluded from model 2. This change has an effect on other parameters in the model, so several other predictors in the two models differ. Several model parameters were largely interchangeable with related parameters that were not included in the final models.

In the models shown in Table 7, the COUNT OF PLOT TREES WITH EARLY *PHYTOPHTHORA* SYMPTOMS could be replaced with related variables such as the count of all trees with *Phytophthora* canker symptoms or a binary variable indicating that trees with *Phytophthora* canker symptoms were present in the plot. Cases were more likely than controls to have other trees with *Phytophthora* canker symptoms in the plot.

Other predictors in the models that are related to plants within the plot were COUNT OF LIVE CALIFORNIA BAY and two similar variables related to the presence of poison oak in the plot. These variables indicate that disease risk is higher in plots that contain poison oak and that risk increases with the number of California bay trees in the plot.

PLOT CANOPY COVER and SKY-EXPOSED CANOPY RATING of the subject tree are related variables. As PLOT CANOPY COVER increases, the subject tree is more likely to be at least partially overtopped and SKY-EXPOSED CANOPY RATING generally decreases. Other variables in the model influence which of these two variables are included in the final models (Table 7). Although SKY-EXPOSED CANOPY RATING is a reasonable predictor of disease in univariate models, PLOT CANOPY COVER is only highly significant in multivariate models, presumably due to interactions with other variables in the models. Overall, disease risk is higher in subject trees with high levels of sky-exposed canopy, and is reduced somewhat in plots with closed canopies. Virtually all of the effect on disease risk of the PLOT CANOPY COVER is associated with closed canopies (>97.5% plot canopy cover). However, because only 3 plots had less than 50% canopy cover, we are unable to meaningfully assess the relationship between very low canopy cover levels and disease risk.

SUM OF STEM CROSS-SECTIONAL AREA may also be related to SKY-EXPOSED CANOPY since larger trees tend to be more dominant in the canopy. This variable was significant in both combined models, but not in the model with tree-variables only (Table 7).

The SWP variable we used was based on the average of the subject tree and additional plot trees because this variable could be computed for all plots, including those in which the subject tree had died. An alternative variable, based on the SWP of the subject tree only, was also highly significant. The effect direction is as described above under univariate models, i.e., disease is associated with relatively low levels of water stress.

Some variables that are correlated with CASE 2001 may actually be indicators of disease rather than factors that increase the risk of infection. The variables *H. THOUARSIANUM* FRUITING PRESENT and BEETLE DAMAGE PRESENT were strongly associated with CASE 2001 in single variable models (Table 6). However, these variables are not entirely independent of the CASE 2001 outcome. The plot assessments for beetle damage and *H. thouarsianum* fruiting include observations of the subject tree, and these symptoms are commonly associated with trees in later stages of the disease. Therefore, we did not include either of these variables in the multivariate models for CASE 2001 or for the DISEASE PROGRESS outcome discussed below.

The variable 2001 CANOPY DIEBACK >20% could also be an early symptom of disease rather than a factor that increases disease susceptibility. Cases are more likely than controls to show elevated levels of canopy dieback. With very few exceptions, the dieback observed in these trees was not the extensive necrosis of the canopy that occurs in the terminal phases of the disease as the crown dies. Rather, dieback in these trees is typically a more subtle, diffuse loss of fine twigs throughout the canopy. The fact that 2001 canopy dieback ratings were more highly significant than 2000 canopy dieback ratings (Table 6) is also consistent with the idea that diseased trees may show a gradual increase in canopy dieback over several years prior to the final rapid canopy collapse. Because diffuse dieback throughout the canopy can

also be associated with agents other than *Phytophthora* spp., this symptom alone is not a definitive indicator of *Phytophthora* canker.

Table 7. Significance levels of parameters in multivariate logistic regression models for the CASE 2001 outcome for coast live oak

Model	Tree variables only	Plot variables only	Combined tree + plot variables model 1	Combined tree + plot variables model 2
	Likelihood ratio Chi square significance level (effect direction)[1]			
Overall model	<0.0001	<0.0001	<0.0001	<0.0001
Predictor variables				
Count of live California bay in plot		0.0009 (+)	0.0070 (+)	0.0014 (+)
Poison oak present			0.0013 (+)	
Poison oak present and shrub cover > 2.5%		0.0064 (+)		0.0021 (+)
Plot canopy cover > 97.5%		0.0305 (-)	0.0004 (-)	0.0093 (-)
Count of plot trees with early *Phytophthora* symptoms		0.0043 (+)	0.0021 (+)	
$SWP_{Plot\ average}$ - $SWP_{location\ max}$ (MPa)	0.0096(+)			
Sum of stem cross-sectional area (cm^2)			0.0002 (+)	0.0033 (+)
More than 2 stems [true]			0.0016 (+)	0.0031 (+)
Number of stems	0.0402 (+)			
Sky-exposed canopy rating	0.0004 (+)			
2001 canopy dieback >20%	0.0088 (+)		0.0093 (+)	0.0091 (+)
AIC	156.35	154.17	135.56	143.50

[1] Effect direction indicates whether outcome is more likely (+) or less likely (-) in the presence of the factor (binary variables) or as the level of the factor increases (continuous variables).

Table 8. Odds ratios of predictor variables in the multivariate logistic regression models shown in Table 7

Model	Tree variables only	Plot variables only	Combined tree + plot variables model 1	Combined tree + plot variables model 2
	Odds ratio (95% confidence limits)			
Predictor variables				
Count of live California bay in plot		115 (5.91 - 4052)	69.4 (2.96 - 2801)	104 (5.36 - 3493)
Poison oak present			4.83 (1.82 - 13.9)	
Poison oak present and shrub cover > 2.5%		4.72 (1.53 - 16.6)		6.10 (1.90 - 22.4)
Plot canopy cover > 97.5%		0.401 (0.165 - 0.919)	0.175 (0.0571 - 0.473)	0.306 (0.116 - 0.752)
Count of plot trees with early *Phytophthora* symptoms		32.1 (2.83 - 537)	64.7 (4.44 - 1428)	
$SWP_{Plot\ average}$ - $SWP_{location\ max}$ (MPa)	39.5 (2.36 - 906)			
Sum of stem cross-sectional area (cm^2)			288 (12.9 - 10840)	57.0 (3.61 - 1305)
More than 2 stems [true]			20.3 (2.94 - 215)	11.4 (2.18 - 93.4)
Number of stems	5.71 (1.08 - 34.1)			
Sky-exposed canopy rating	14.7 (3.17 - 80.4)			
2001 canopy dieback >20%	2.99 (1.31 - 7.09)		3.63 (1.36 - 10.5)	3.39 (1.35 - 9.09)

Number of stems

The number of stems and the derived binary variables MULTISTEMMED and MORE THAN 2 STEMS were predictors of the case outcome in both univariate and multivariate logistic models for coast live oak in both 2000 (Swiecki and Bernhardt 2001a, 2002) and 2001 (Tables 7, 8). Even after adjusting for other factors that affect disease risk, multistemmed coast live oaks were more likely to show *Phytophthora* symptoms than single-stemmed trees. In order to explore this relationship in more detail, in 2001 we collected disease data for individual stems of subject trees and additional trees used for water potential readings. This allowed us to calculate both apparent stem infection rates and tree infection rates for trees with varying numbers of stems. Infection rates are inferred on the basis of symptomatic stems or trees and are therefore equivalent to the proportion of symptomatic units (stems or trees). For convenience, we use the term "infection rate" in this discussion rather than "proportion of symptomatic units".

A higher infection rate among multistemmed trees can be explained on the basis of probability alone if multiple stems represent multiple independent sites where infection may occur. If we calculate the apparent *Phytophthora* infection rate on a stem basis, we can estimate the probabilities that a given multistemmed tree will have one or more infected stems under the null hypothesis that each stem has the same likelihood of being infected. The observed and calculated expected values under this hypothesis are presented in Table 9. We used the apparent stem infection rate for each stem count class to calculate the expected values, which produces more conservative expected values than either the overall stem infection rate or the infection rate for single-stemmed trees.

Under the null hypothesis, infection in one stem is independent of infection in other stems (i.e., being multistemmed per se does not affect the likelihood of infection). Under this hypothesis, the expected tree infection rate increases with number of stems because the chance of having at least one infected stem goes up as the stem count increases (Table 10). The overall expected tree infection rates calculated under the null hypothesis for trees with varying numbers of stems (Table 10) do not differ significantly from observed tree infection rates (Chi-square test). However, as shown in Table 9, the number of trees showing multiple infected stems departs from expectations. For example, for a tree with 3 stems, the probability that all 3 stems will be symptomatic under the null hypothesis is $(0.476)^3=0.108$ whereas the observed rate was 0.286. For both trees with 2 and 3 stems, Chi-square tests indicate that the observed distributions of trees with varying numbers of infected stems (Table 9) differed significantly from the expected distributions (Chi-square $P<0.001$ and $P=0.041$, respectively).

It appears likely that tree factors (including genetic predisposition or resistance) and other factors (such as local inoculum production) increase the likelihood that multiple stems from the same tree will show the same disease outcome. This would lead to larger than expected numbers of multistemmed trees with either no infected stems or all stems infected, as we observed (Table 9). Further investigation of this effect and possible partitioning into genetic and non-genetic components would require larger numbers of multistemmed trees than are included in this study.

Table 9. Observed and expected incidence of *Phytophthora* symptoms on individual stems and trees overall for single-stemmed and multistemmed coast live oaks.

Stems per tree	Observed stem infection rate	Number of infected stems/tree	Observed tree count	Observed percent in class	Expected tree count[1] in class	Expected percent[1] in class
1	31.3%	0	77	68.8%	--	--
		1	35	31.3%	--	--
2	34.2%	0	22	57.9%	16.4	43.3%
		1	6	15.8%	17.1	45.0%
		2	10	26.3%	4.4	11.7%
3	47.6%	0	4	28.6%	2.0	14.3%
		1	4	28.6%	5.5	39.2%
		2	2	14.3%	5.0	35.6%
		3	4	28.6%	1.5	10.8%

[1]Expected values are calculated from a binomial expansion using the observed stem infection rate for each class of multistemmed trees under the null hypothesis that stem infections in a given tree are independent of each other.

Table 10. Overall observed and expected frequency of *Phytophthora* symptoms on coast live oak trees with varying numbers of stems. Trees are symptomatic if at least one stem has *Phytophthora* symptoms.

Stems per tree	Number of trees	Observed percent symptomatic trees	Expected percent symptomatic trees[1]
1	112	31.3%	--
2	38	42.1%	52.7%
3	14	71.4%	67.5%

[1]Expected values are calculated from a binomial expansion using the observed stem infection rate for each class of multistemmed trees under the null hypothesis that stem infections in a given tree are independent of each other.

DISEASE PROGRESS outcome

For the DISEASE PROGRESS outcome, we fitted a logistic regression model to a subset of the data that includes only the cases rather than both cases and controls. The models apply to disease progress in trees in which cankers have already been initiated. The overall sample size for this outcome is lower than that used for the CASE 2001 outcome. This tends to reduce the number of significant predictors in the models.

Univariate models. Most of the individual variables related to disease progress were not significant predictors of disease occurrence and vice versa. Only variables related to tree size (SUM OF DBH, SUM OF STEM CROSS-SECTIONAL AREA) and the variables noting the presence of late disease symptoms in the plot (beetle damage and *H. thouarsianum* fruiting) were significantly associated with both CASE 2001 and DISEASE PROGRESS in univariate models (Table 6). For reasons discussed above, we did not use the variables *H. THOUARSIANUM* FRUITING PRESENT and BEETLE DAMAGE PRESENT when constructing multivariate models for DISEASE PROGRESS.

Multivariate models. Unlike the CASE 2001 model, few parameters were significant in the DISEASE PROGRESS model. The best multivariate logistic regression model for the DISEASE PROGRESS outcome contained only two parameters (Table 11). Model fit was not very good.

Significant two-variable models could be developed using MADRONE REGENERATION PRESENT and either SUM OF DBH or COUNT OF LIVE OVERSTORY TREES OTHER THAN BAY. However, SUM OF DBH was not significant if included in the same model with COUNT OF LIVE OVERSTORY TREES OTHER THAN BAY. This latter variable was negatively correlated with disease progress. Stem DBH and plot tree density are related in that they are both indicators of competition or the relative suppression of the subject

tree. As the number of overstory trees in the plot increases, subject trees are exposed to greater levels of competition for light, nutrients, and water, leading to reduced growth and smaller stem diameters. Therefore, one possible interpretation of these model parameters is that disease progress was less likely to occur in suppressed trees, i.e., trees with many overstory competitors and/or small diameters. Because the count of bay trees is positively associated with disease, excluding bay from the total overstory density provides a measure of competition without the complicating factor that high bay density may be associated with increased inoculum density.

Although no madrone-related variables were significant predictors of the CASE 2001 outcome and the number of madrone trees in the plot was not a significant predictor of DISEASE PROGRESS, two other madrone-related variables were predictors of DISEASE PROGRESS. The variable MADRONE REGENERATION PRESENT was a better predictor of the DISEASE PROGRESS outcome than the related variable MADRONE OVERSTORY (Table 6), and was the only significant madrone variable in multivariate models. Disease progress was less likely to occur in plots where madrone regeneration was present. Madrone regeneration and overstory are also inversely correlated with coast live oak regeneration variables, which were weaker predictors of the DISEASE PROGRESS outcome (Table 6). Overall, sites with madrone overstory or regeneration had significantly lower populations of coast live oak regeneration than plots that lack these factors (t-test p=0.0005 and 0.0232, respectively).

Table 11. Multivariate logistic regression model[1] parameter estimates and model fit for the DISEASE PROGRESS outcome for coast live oak cases only.

Predictor variables	Likelihood Ratio Prob>χ^2	Effect direction	Odds ratio (95% confidence interval)
Count of live overstory trees other than bay	0.0056	-	0.0082 (0.000096 - 0.276)
Madrone regeneration present [true]	0.0181	-	0.189 (0.0407 - 0.756)
AIC	67.09		

[1]Overall model likelihood ratio p=0.0009, n=56.

Changes in disease status of tanoak subject trees

In 2001, one tanoak was reclassified from a case to a control tree based on a reevaluation of symptoms. Two tanoaks classified as controls in 2000 were symptomatic in 2001. These changes shifted the overall balance of subject trees to 12 controls and 10 cases in 2001. Six of these 10 tanoak cases showed an increase in disease severity (Figure 6) and most of these also showed evidence of recent bleeding from cankers.

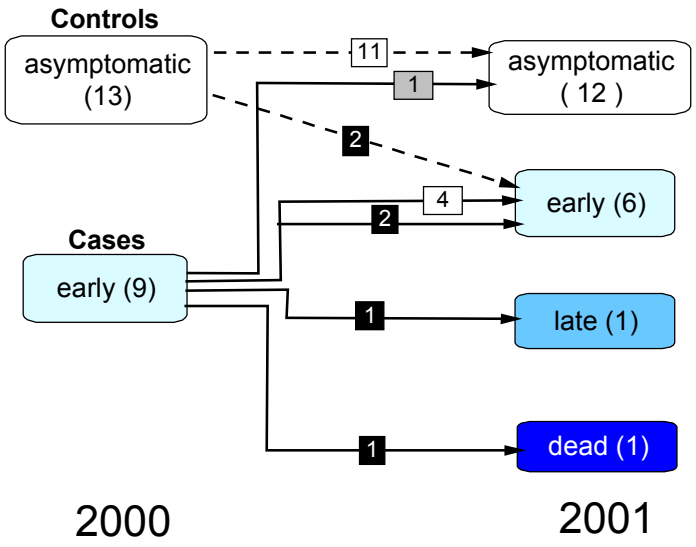

Figure 6. Diagram of transitions related to disease progress in tanoak subject trees from September 2000 to September 2001. Numbers represent counts of subject trees. White rectangles along arrows represent trees with no apparent change in condition. Black rectangles along arrows represent trees showing increased severity of *Phytophthora*-related symptoms. Gray rectangle indicates tree reclassified as asymptomatic relative to *Phytophthora*-related symptoms.

Factors associated with disease in tanoak

Because of the low sample size for tanoak, our ability to develop meaningful logistic regression models for the CASE 2001 outcome is limited and modeling of the DISEASE PROGRESS outcome is not possible. The total sample for tanoak can be increased from 22 trees (10 cases, 12 controls) to 29 trees by including the seven additional trees used for water potential measurements and using the presence of *Phytophthora* symptoms as the outcome (12 symptomatic, 17 asymptomatic). Regardless of the outcome used, very few predictors are significant in either univariate or multivariate models.

The variable 2000 CANOPY DIEBACK RATING was a significant predictor of disease status in 2001 ($p=0.0111$). Canopy dieback ratings in 2001 were also positively associated with disease. As noted in the above discussion for coast live oak, canopy dieback could be related to the girdling effect of cankers on the main stem(s), and therefore represent a symptom related to the CASE 2001 outcome. However, tanoak twigs and leaves may also be infected by *P. ramorum*, whereas bole cankers are the only known direct symptoms associated with *P. ramorum* in coast live oak. Hence, for tanoak, canopy dieback could represent a distinct phase of the disease which may be a precursor to the development of cankers on the main stem.

The only other significant predictor of *Phytophthora* symptoms in 2001 was OTHER DECLINE. Trees rated as having symptoms of decline due to other agents were less likely to have *Phytophthora* symptoms. The OTHER DECLINE variable was significant in both a univariate model (likelihood ratio $p=0.0298$) and in a two-variable model (overall model $p=0.0009$, $n=29$) that included both OTHER DECLINE (likelihood ratio $p=0.0059$) and 2001 CANOPY DIEBACK (likelihood ratio $p=0.0016$).

No other variables that we tested were significant predictors of the CASE 2001 outcome for tanoak. Although plot insolation had been a significant predictor of the CASE 2000 outcome (Swiecki and Bernhardt 2001a), it was not a significant predictor of CASE 2001 or of the *Phytophthora* disease outcome for subject trees plus additional trees.

Disease and disease progress in plot (non-subject) trees

Disease in plot trees

In addition to the subject tree at the center of each plot, we also collected disease data on the 523 coast live oaks and 163 tanoak trees in the plots. This total includes 60 coast live oaks and 31 tanoaks that were dead at the time that plots were established in 2000. The overall health of coast live oak and tanoak plot trees in September 2001 is shown in Figure 7. The total number of plot trees of these species is slightly less than previously reported (Swiecki and Bernhardt 2001a) because some trees included in the 2000 plot totals were found to be slightly outside the 8 m plot radius upon more precise remeasurement in 2001.

In 2001, the percentage of plot trees with *Phytophthora* canker symptoms was higher overall for tanoak (35%) than for coast live oak (20%, Figure 7). For coast live oak, the incidence of symptomatic trees was significantly higher in case plots than in control plots ($P<0.0001$, Fisher's exact test), but for tanoak, the incidence of symptomatic trees did not differ significantly between case and control plots.

Apparent changes in the health of tanoaks and coast live oaks between September 2000 and September 2001 are graphed in Figure 8. Among trees with symptoms of *Phytophthora* infection in September 2000, 30% (25/83) of coast live oaks and 47% (15/32) of tanoaks progressed to a more severe symptom class (e.g., early to late, late to dead) by September 2001. Mortality between September 2000 and September 2001 among trees with *Phytophthora* symptoms in 2000 was 6% (5/83) for coast live oak and 19% (6/32) for tanoak. In contrast, mortality rates in trees lacking *Phytophthora* symptoms in 2000 were 1% (4/380) for coast live oak and 0% (0/100) for tanoak. These mortality rates are significantly lower than rates observed in symptomatic trees ($p=0.0115$ and $p<0.0001$, Fisher's exact test, for coast live oak and tanoak, respectively).

The appearance of new *Phytophthora* symptoms was more common in tanoak than in coast live oak. Among plot trees that were asymptomatic in September 2000, symptoms of *Phytophthora* canker were noted in 2001 in 1.6% (6/380) of the coast live oaks and 8% (8/100) of the tanoaks (Figure 8). Conversely, 8 coast live oaks and 9 tanoaks that had shown evidence of bleeding cankers in 2000 were apparently asymptomatic in 2001. These may represent trees in which disease has become quiescent, gone into remission, and/or trees that were misclassified due to bleeding associated with causes other than *Phytophthora*. We reassigned these trees to the asymptomatic or the other decline symptom classes as appropriate. These new designations are reflected in Figures 7 and 8.

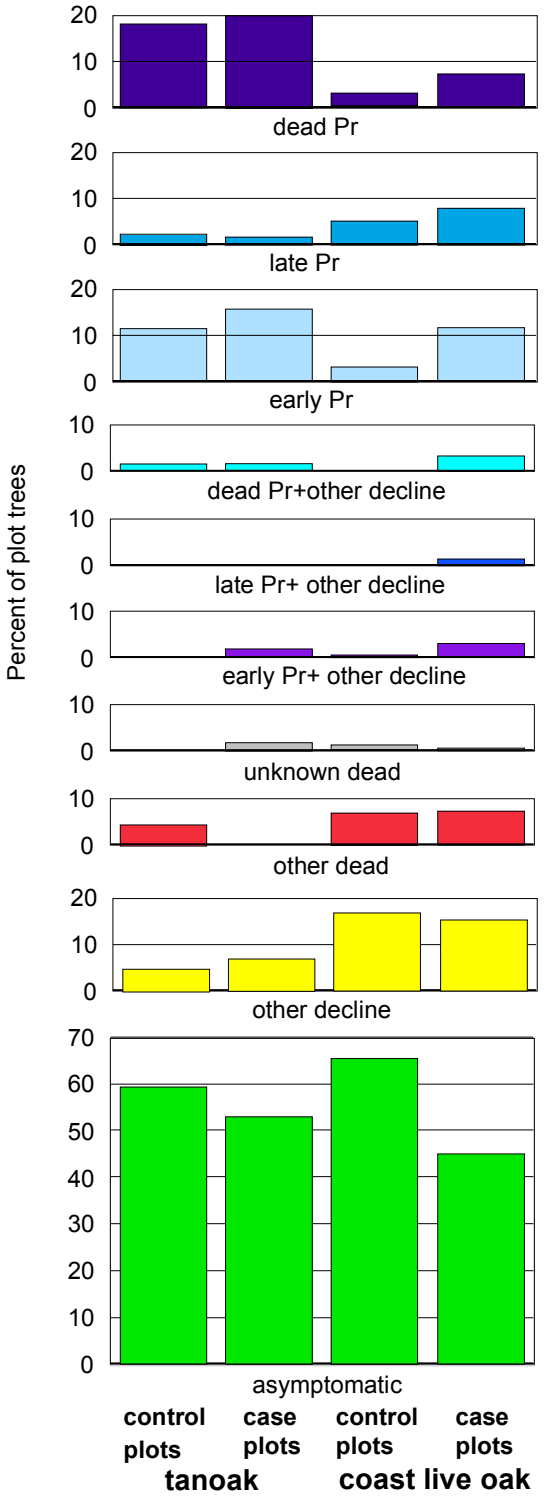

Figure 7. Health status in September 2001 of nonsubject tanoak (left two columns) and coast live oak (right two columns) in control or case plots. Tanoak trees in control plots=91, tanoak trees in case plots=72. Coast live oak trees in control plots=294, coast live oak trees in case plots=229. Subject trees (at each plot center) are excluded from these figures. Pr = *Phytophthora* canker symptoms

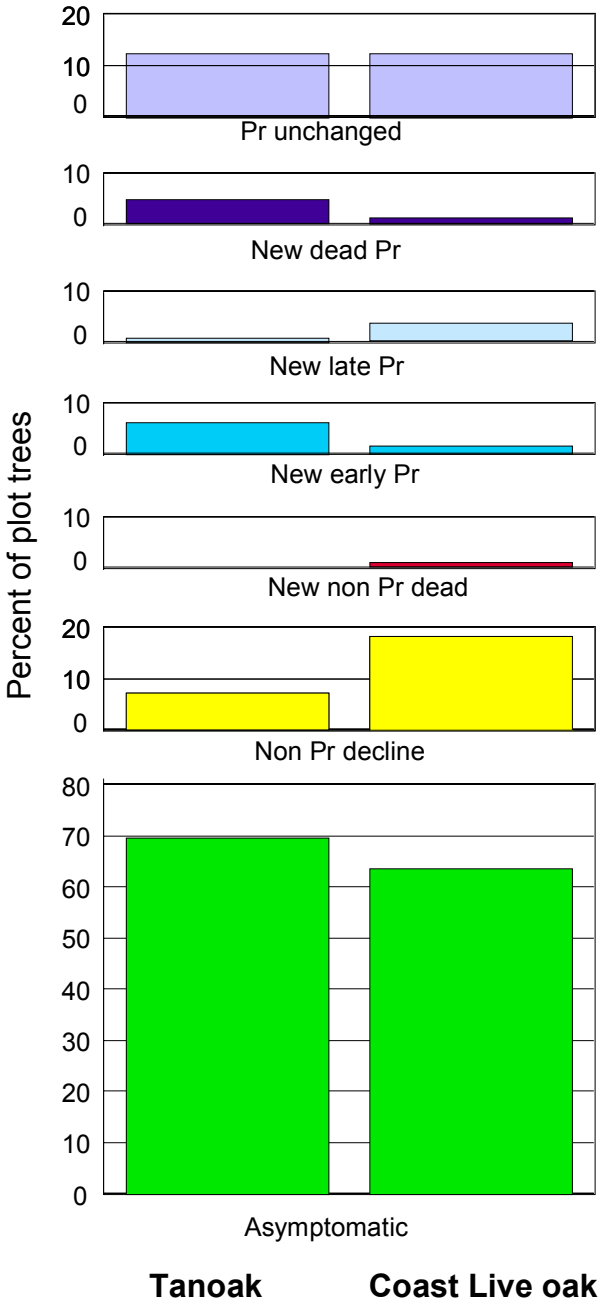

Figure 8. Disease progress to September 2001 of nonsubject tanoak (n=132), left column, and coast live oak trees (n=463), right column, alive in September 2000. Trees showing both *Phytophthora* (Pr) symptoms and decline symptoms due to other agents are included in the *Phytophthora* categories.

Factors associated with disease in plot trees

For both coast live oak and tanoak plot trees, the number of plot trees observed with both *Phytophthora* symptoms and decline or mortality due to other agents (Figure 7) was only about half of what would be expected if these two factors were independent of each other. For coast live oak only, this deviation from expected values was significant (P=0.0089, Fisher's exact test). At least for coast live oak,

Phytophthora canker appears to be less likely to occur in trees that are in severe decline due to other factors or agents.

For both coast live oak and tanoak, plot trees with more than 2.5% sky-exposed canopy were significantly more likely to have *Phytophthora* canker symptoms than trees with less exposure to overhead light (p=0.0004 and p=0.0010, for coast live oak and tanoak respectively, Fisher's exact test). Coast live oak trees with less than 2.5% sky-exposed canopy were also more likely than trees with more sky exposure to be rated as declining or dead due to causes other than *Phytophthora* (p<0.0001, Fisher's exact test), but no such relationship was observed for tanoak.

Logistic regression models

Coast live oak. We constructed multivariate logistic regression models for coast live oak plot trees using the presence of *Phytophthora* canker symptoms as the outcome. The final model (Table 12) includes several variables that are the same as or closely related to variables in the subject tree models. The tree-related variable SKY-EXPOSED CANOPY >2.5% in the plot tree model is a variant of the SKY-EXPOSED CANOPY RATING variable that was significant in some subject tree models. PLOT CANOPY COVER and COUNT OF LIVE CALIFORNIA BAY IN PLOT were also significant in both plot tree (Table 12) and subject tree models (Tables 7, 8). As was the case for subject trees, *H. THOUARSIANUM* FRUITING PRESENT and BEETLE DAMAGE PRESENT were highly significant if included in the plot tree model but we excluded these variables from our final model for the same reasons discussed earlier. The plot mean SWP (i.e., mean of subject tree and additional plot trees, if any) was significant at p=0.0815 but was not included in the model in Table 12.

Two variables in the plot tree model do not have counterparts in the subject tree model. Decline due to agents other than *Phytophthora* was not a significant predictor of the subject tree CASE 2001 outcome even though it was a significant predictor of *Phytophthora* canker in plot trees. Although the percentage of subject trees rated as in decline due to other agents (15.6%) was close to percentage for plot trees (18.3%), it is possible trees in severe decline were less likely to be selected as subject trees due to a lack of leaves available for SWP measurements. This possible selection bias might account for the fact that decline due to other agents was not significant for subject trees but was for plot trees.

Study location was also highly significant in the plot tree model, indicating that disease risk among plot trees varies by location. This suggests that some location-specific variables that were not measured for plot trees may influence disease risk. At least some of these variables could be the same as or related to other significant variables in the subject tree models. For example, the average number of stems per subject tree varies by location (F test p=0.0003). Although we did not record the number of stems for all plot trees, if the number of multistemmed plot trees also varies by location, this factor might account for at least some of the location effect. Location was not a significant predictor for the subject tree outcome CASE 2001, and is not really relevant in that model because case and control trees were intentionally selected in similar ratios at each location.

Tanoak. The multivariate model for tanoak plot trees had only three variables (Table 12) and fit was relatively poor. Although this is not an especially robust model, it is interesting to note that the variables in the model are all variables that were identified as predictors of the CASE 2001 outcome for coast live oak subject trees, although the effect direction for SWP is the reverse of that seen for coast live oak. Again, the *H. THOUARSIANUM* FRUITING PRESENT and BEETLE DAMAGE PRESENT variables were highly significant in both univariate and multivariate models, but were not included in the final model because they are essentially related outcomes rather than predictors. The OTHER DECLINE variable could also be included in the multivariate model (likelihood ratio p=0.0912) but adding this variable did not improve fit as measured by the AIC statistic.

Table 12. Significance of parameters for multivariate logistic regression models for the presence of *Phytophthora* symptoms in non-subject plot trees for coast live oak and tanoak.

	Coast live oak[1]		Tanoak[2]	
	Likelihood Ratio Prob>χ^2 (effect direction)	Odds ratio (95% confidence interval)	Likelihood Ratio Prob>χ^2 (effect direction)	Odds ratio (95% confidence interval)
Overall model	<0.0001		0.0001	
Predictor variables				
Sky exposed canopy >2.5% [true]	0.0097 (+)	2.46 (1.23 - 5.20)	0.0034 (+)	3.14 (1.46 - 6.87)
Tree decline due to other agents [true]	0.0204 (-)	0.464 (0.231 - 0.890)		
Count of live California bay in plot	<0.0001 (+)	63 (10.6 - 433)		
Plot canopy cover rating	0.0322 (-)	0.219 (0.0538 - 0.878)		
Study location (as random effect)	<0.0001	range: 0.0035 to 63		
2001 $SWP_{plot\ average}$			0.0371 (-)	0.209 (0.0455 - 0.911)
Poison oak present			0.0362 (+)	2.88 (1.07 - 8.10)
AIC	423.3		198.3	

[1]n=523
[2]n=163

DISCUSSION

The purpose of the models presented above is to identify factors that predict disease occurrence and to assess the relationships between these factors and disease. The study design does not allow us to produce models that can be used to predict disease levels in a stand.

Both the disease occurrence and disease progress models are likely to contain only a subset of the variables that affect disease risk. For example, climate factors that may have influenced disease development are not explicitly considered in these models, although SWP measurements are correlated with a number of climate-related factors. By restricting our study locations to areas where disease is common, we know that climate factors must be reasonably favorable for disease development. However, climate variables are likely to affect disease risk across the range of the disease, and may interact with variables identified in this study.

Disease occurrence

The logistic models associating disease with plot and tree risk factors that we developed using 2001 data were similar to those based on data collected in 2000 (Swiecki and Bernhardt 2001a, 2002). Some of the differences between the 2000 and 2001 models are associated with the fact that a small number of trees were reclassified from asymptomatic to symptomatic or vice versa. Other differences are associated with the fact that some of the additional variables had not been measured in 2000.

Canopy dieback was significant in both 2000 and 2001 models for both coast live oak and tanoak. As noted above, dieback is probably an early indicator of disease rather than a factor that increases the likelihood that a tree will become infected. Diffuse canopy dieback has not been generally recognized as an early symptom of *Phytophthora* canker in naturally-infected trees, although it is not unexpected that this symptom would develop in trees that have been partially to mostly girdled by stem cankers. In artificially-inoculated coast live oak and tanoak trees growing in natural stands, Rizzo and others (2002b) noted that crown thinning, presumably resulting from diffuse canopy dieback, developed by 9 months after inoculation.

The presence of other host trees with *Phytophthora* canker within the plot was also previously identified as a factor correlated with disease in coast live oak subject trees (Swiecki and Bernhardt 2001a,

2002). As we noted in those previous reports, the significance of this variable indicates that spatial clustering of disease occurs on the scale of the plot area (0.02 ha). Based on our current understanding of disease epidemiology, it does not appear that any substantial disease spread occurs from infected oaks (Swiecki and Bernhardt 2001a, 2002) although this question has not yet been fully resolved (Davidson and others 2002). Therefore, the presence of other symptomatic trees within a plot is probably related to other local factors (e.g., inoculum densities, infection period dynamics, host genetic makeup, host stress levels) that directly influence the probability that a tree will become infected and develop symptoms.

At least one factor identified in both 2000 and 2001 models appears to be related to local inoculum density. Following our original report (Swiecki and Bernhardt 2001a), California bay was identified as one of a number of native species that are foliar hosts of *P. ramorum* (Davidson and others 2002, Rizzo and others 2002a). Because no other known hosts of *P. ramorum* were significant predictors of disease, our analyses suggest that California bay may be one of the most important natural sources of inoculum, at least in coast live oak stands similar to those in our study. *P. ramorum* forms both sporangia and chlamydospores in abundance on California bay leaves (Davidson and others 2002). California bay may be an especially important host because it is evergreen and therefore has susceptible leaf tissue available for colonization and sporangium production throughout the winter rainy season.

Although other known overstory and understory hosts of *P. ramorum* were tested in our 2001 models, the only other plant species variable that surfaced as a significant predictor of disease was poison oak presence. Poison oak has not been identified as a host to date, although this species has not been screened as intensively as others due to problems inherent in its handling. If poison oak is a host of *P. ramorum*, the association with disease risk could also be related to inoculum production on this species. However, because poison oak is deciduous, its importance as a source of inoculum might be restricted to years when significant rains continue into late spring. If poison oak is not a host of *P. ramorum*, poison oak presence may simply be correlated with site conditions or other factors that influence disease development.

Several other variables in the disease risk models could be related to inoculum interception by host trees. One explanation for the significance of the related canopy cover, sky-exposed canopy, and stem cross-sectional area variables could be that more dominant and/or more exposed trees intercept airborne inoculum more efficiently than overtopped trees. This possible explanation presupposes that airborne inoculum density is greater in the upper portions of canopy than within the canopy. Another possibility is that tree size is the major factor influencing inoculum interception. Larger trees may intercept more inoculum and concentrate it via stem flow during rain events, increasing their risk of disease.

However, these and other variables could also be explained through an alternative hypothesis unrelated to inoculum interception: trees with low vigor may have a lower risk of becoming infected than faster-growing, more vigorous trees. The list of significant predictors of disease that are rather directly related to tree growth rate and vigor include not only canopy cover, sky-exposed canopy, and stem cross-sectional area, but also SWP and decline associated with other agents. For all of these variables, the conditions that are associated with greater tree growth and vigor (large diameter, more exposed canopy/lower plot canopy cover, high SWP/low water stress, lack of severe impacts from other decline agents) are also correlated with greater risk of *Phytophthora* canker.

P. ramorum primarily attacks phloem tissues in the bark of coast live oak and apparently infects intact, non-wounded tissues. Faster-growing coast live oak trees could be more susceptible to infection than slow-growing trees due to differences in characteristics of the outer bark, such as more or larger bark fissures. Alternatively, the phloem in more stressed trees may be less favorable for *P. ramorum* colonization due to differences in thickness, physiological status (e.g., starch content), and/or other properties. Studies with artificially-inoculated trees have shown substantial tree-to-tree variability in the infection rates of intact bark (J. Davidson, personal communication) and the expansion of cankers in wound inoculations (Rizzo and others 2002b), which suggests that various host factors may influence pre- and post-penetration disease development.

Disease risk models based on plot tree data were fairly similar to models based on subject trees, suggesting that variables such as canopy exposure, canopy cover, and bay density are relatively robust

predictors of disease. Furthermore, sky-exposed canopy and decline associated with other agents showed similar relationships with *Phytophthora* canker in a cross-sectional field study in Sonoma County (Swiecki and Bernhardt 2001b). This increases our confidence that many of the stronger effects in the models are likely to be associated with disease risk in areas beyond where our plots are located.

To date, our assignment of trees as cases or controls has been based almost exclusively on field symptoms. Particularly when cankers are small and/or not actively bleeding, some uncertainty is associated with the assignment of disease status. Although the disease status of a few of the study trees has been confirmed by isolation, we have not yet conducted a wider sampling of subject trees for several reasons. Since repeated observations are made on these trees, we wanted to avoid damaging trees through sampling. As the technology and methodology for detecting *P. ramorum* and other *Phytophthora* species in canker tissue improves, we have anticipated that it will eventually be possible to conduct minimally-invasive sampling of subject trees and use DNA-based assays to determine which pathogen(s) may be associated with canker symptoms in study trees. Finally, under the initial assumption that disease would progress in infected trees, we anticipated that questions about disease status would be resolved through followup observations. As discussed below, it is not certain that this last assumption is entirely valid.

If the disease status of the study trees could be reliably confirmed without influencing disease progress, some further refinement of our disease risk models may be possible. In particular, identification of the pathogen(s) associated with study trees would clarify whether the models apply to *P. ramorum* canker or a mixture of cankers caused by *P. ramorum* and other *Phytophthora* species, such as the *P. ilicis*-like organism (Rizzo and others 2002b) .

Disease progress

Between September 2000 and September 2001, visible disease progress was evident in the majority of all coast live oak (Figures 4, 5) and about half the tanoak (Figure 6) cases. Recent sap bleeding from cankers was more common in trees where disease progress had occurred in the past year, but this symptom was not consistently associated with disease progress. The transition from early symptoms (bleeding cankers only) to late symptoms (cankers plus beetle damage and/or *H. thouarsianum* fruiting) occurred in a minority of the cases (Figures 4, 5, 6) and plot trees (Figure 8) for both coast live oak and tanoak. *Phytophthora*-related mortality in both cases and plot trees was higher for tanoak than for coast live oak, and was well above mortality rates associated with other agents over this period. Nonetheless, the proportion of symptomatic trees that died within the one year observation period represented only a fraction of the symptomatic trees (Figures 4, 6, 8). If rates of disease progress in infected trees remain relatively constant, some currently symptomatic tanoaks may survive for several more years and some symptomatic coast live oaks could be expected to survive much longer. However, it remains to be seen whether disease progresses in a consistent or variable fashion in symptomatic trees.

A substantial fraction of the cases showed no visible evidence of disease progress over the observation period and cankers in some trees appeared to be dried out and inactive. Further monitoring is necessary to determine whether these represent infections in which the pathogen has died out or quiescent infections that may reactivate under more favorable conditions. Differences in disease aggressiveness could be associated with a variety of factors, including the possibility that multiple species of *Phytophthora* are involved. We must also consider the possibility that some apparently inactive cankers have continued to expand but that outwardly visible symptoms have not appeared. In tanoak especially, infected trees sometimes display few external symptoms of disease prior to death (D. Rizzo, personal communication).

Rates of symptom progression and mortality that we observed in subject and plot trees are comparable to levels reported by McPherson and others (2002) for the period March 2000 through March 2001. Both we and McPherson and others (2002) observed few newly symptomatic trees, with a higher rate of newly symptomatic trees among tanoak than coast live oak (Figure 8). McPherson and others (2002) suggested that the presence of newly symptomatic trees implied that new infections are still occurring. However, an alternative hypothesis is equally likely, i.e., that no new infections have occurred

for several years and that newly symptomatic trees represent slow-growing cankers that were not evident in previous years. Infected trees sometimes lack external evidence of cankers, the sap bleeding symptom is variable over time, and the rate of disease progress is apparently variable among symptomatic trees. Hence, it seems likely that some trees may remain free of obvious symptoms for an extended period after being infected, i.e., the apparent latent period may be variable within a cohort of trees that were infected at a given point in time.

The disease progress model for coast live oak is preliminary in that it is based on disease progress over one year only on a limited number of trees. Furthermore, disease progress could have occurred in some trees without obvious advancement in visual symptoms. Nonetheless, several aspects of this preliminary model are of interest. First, other than beetle damage and *H. thouarsianum* fruiting, only tree diameter (or cross-sectional area) was related to both disease risk and subsequent disease progress. It is possible that for coast live oak, factors associated with the production or interception of inoculum (e.g., California bay density, sky-exposed canopy/plot canopy cover, and presence of multiple stems) are not strongly related to the subsequent progress of disease in trees that are already symptomatic. This would be the case if disease progress is primarily related to canker expansion rather than the development of new cankers. Such a pattern would be expected if infection periods occur infrequently, such as only in years with favorable rainfall regimes.

It seems unlikely that the presence of madrone regeneration per se has any direct influence on disease progress in infected coast live oaks. The negative association between madrone regeneration and disease progress is more likely related to site factors that were not represented in our analyses. These site conditions may tend to promote madrone regeneration and disfavor disease progress and coast live oak regeneration (Table 6). Some site factors that have been associated with madrone regeneration include soil disturbance, bare mineral soil or soil low in organic matter, and some kinds of canopy gaps (McDonald and Tappeiner 1990). The other significant variable in the model (number of overstory trees other than bay) could be interpreted to suggest that disease progress is slower in coast live oaks that are somewhat suppressed due to competition.

The relatively poor fit of the disease progress model may in part be due to the small number of explanatory variables that were available for model development. However, it is also possible that some trees nominally at higher risk for disease progress are resistant due to genetic characteristics or other unquantified factors, leading to poor prediction of the disease progress outcome. An improved disease progress model may be useful for identifying specific trees that may be of interest for additional genetic studies.

Water relations

We observed a strong correlation between September SWP measurements made on individual trees in 2000 and 2001. 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.

Coast live oaks that maintain high SWP levels through the end of the growing season apparently have a greater risk of developing *Phytophthora* canker symptoms than do more water-stressed trees. As noted previously (Swiecki and Bernhardt 2001a, 2002), this finding was the opposite of the effect that we had initially anticipated. However, our observations have been made during two normal to dry rainfall years. Differences between wetter and drier plots could have been more pronounced in these two years than might be the case in years with above average rainfall. Because we do not know whether SWP differences would have been significant in wetter years that might have coincided with the initial infection of these trees, we cannot determine whether the relationship between disease risk and SWP is more related to pre- or post-infection processes.

Another somewhat counterintuitive finding is that symptomatic trees have not shown a general decline in SWP as symptoms have progressed. SWP appears to remain relatively unchanged in

symptomatic trees until the tree reaches the final stage of disease in which the top dries out rather rapidly. This latter phenomenon seems to be the only part of the disease that corresponds with the popular term "sudden oak death". Because *P. ramorum* cankers affect the bark but generally do not affect substantial amounts of xylem tissue (Rizzo and others 2002b), water transport to the top is apparently not strongly affected by these cankers. This phenomenon allows us to use SWP as an indicator of levels of plant water stress that the tree would have experienced irrespective of its disease status. It also appears that destruction of the sapwood by *H. thouarsianum* and/or other agents must also pass a critical threshold before SWP is affected. Small losses in water transport due to limited amounts of sapwood destruction may be compensated for through the loss of leaf area via the diffuse crown dieback that is associated with disease.

The fact that trees with *Phytophthora* cankers do not show a gradual increase in water stress over the period of at least one year reduces the chance that remote sensing of water stress signatures will be useful for early detection of disease. Our results suggest that severe water stress in trees with *Phytophthora* canker is likely to show up in the canopy only when death of the top is imminent. Furthermore, water-stressed coast live oaks appear to be at a reduced risk for disease, so many trees that show water stress in late summer are likely to be free of *Phytophthora* infection. The diffuse dieback symptom may provide a more reasonable target for the early detection of symptomatic trees, although such dieback could also be associated with other diseases or might be mimicked by symptoms such as oakworm damage in remotely sensed data.

We were interested to see that the decrease in SWP was relatively uniform across all study locations and for both species. If confirmed by additional readings, this raises the possibility that early fall monitoring of SWP of certain species in selected locations could be used as an index of regional drought-stress severity.

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