Evaluation of stem water potential and other tree and stand variables as risk factors for *Phytophthora* canker development in coast live oak and tanoak

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SUMMARY

We conducted a case-control study to examine the role of water stress and various other factors on the development of *Phytophthora* sp. nov. cankers 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, we collected data in 150 circular plots (8 m radius) in areas where disease caused by *Phytophthora* sp. nov. is prevalent. Each plot was centered around a subject case or control tree. We collected data at 10 locations in Marin County, and 1 location each in Sonoma and Napa Counties. Subject tree variables, data on host and nonhost trees in the plot, and other plot characteristics were fitted into logistic regression models.

For *Q. agrifolia*, the incidence of decline and death related to *Phytophthora* was almost equal to rates of decline and recent mortality due to other agents. For *L. densiflorus*, decline and mortality related to *Phytophthora* was far more common than decline and mortality due to other agents. *Phytophthora* infection in plot trees other than the subject (case or control) tree was more common in case plots than in control plots. This result indicates that *Phytophthora* infected trees are spatially aggregated, at least on the scale of the plot size used in this study (0.02 ha). However, counts of plot trees with advanced symptoms of decline due to *Phytophthora* canker were not associated with disease in the subject tree. Furthermore, within plots, counts of trees with early and advanced symptoms of *Phytophthora* canker were not correlated. Thus, our data do not indicate that inoculum produced within the plot plays a clear role in the spatial aggregation of diseased trees.

Midday stem water potential (stem Ψ) in *Q. agrifolia* subject trees ranged from -0.25 to -3.1 MPa. Stem Ψ was higher (indicating lower water stress) in cases than controls. Furthermore, the amount of trunk girdling caused by *Phytophthora* cankers also increased as stem Ψ increased. These results indicate that *Q. agrifolia* trees in moister sites are at a higher risk for disease than are those in drier sites. Other variables significantly associated with disease in *Q. agrifolia* include tree canopy dieback and the amount of the canopy shaded by other trees.

Stem Ψ in *L. densiflorus* subject trees ranged from -0.425 to -1.65 MPa. No significant relationships between stem Ψ and disease were evident for *L. densiflorus*, but relatively few *L. densiflorus* subject trees were included in the study.

INTRODUCTION

A newly recognized pathogen (an as yet unnamed *Phytophthora* sp. nov.) has been associated with elevated levels of mortality in tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*), and California black oak (*Q. kelloggii*) trees in a number of California coastal counties over the past few years (Garbelotto et al 2001). Early symptoms of the disease, which is commonly referred to as "sudden oak death", include bleeding bark cankers on the bole. The sapwood-decaying fungus *Hypoxylon thouarsianum*, oak bark beetles (*Pseudopityophthorus* spp.), and ambrosia beetles (*Monarthrum* spp.) are commonly associated with *Phytophthora*-infected trees in later stages of decline. Tanoak trees exhibiting symptoms consistent with the syndrome were reported in Marin County, CA in 1995(Svihra 2001). By the time that this study was conducted in fall 2000, *Phytophthora*-related tanoak and oak mortality had become widespread in portions of Santa Cruz, Marin, Monterey, Napa, and Sonoma Counties.

Although the epidemiology of this newly recognized disease has not yet been determined, the pattern and distribution of the affected trees in the field suggest that a number of factors may affect the risk of disease development. In particular, water stress has 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 et al 1987) and is also a predisposing factor for *Hypoxylon* infection (Sinclair et al 1987) and beetle attack.

To examine the role of water stress and several 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 (or case referent) 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 condition (referred to as the case group) is compared with a second group of subjects that do not exhibit the condition (the control group). Factors preceding the outcome (i.e., disease development) 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; direct causality cannot be shown from a case-control study. Nonetheless, the study design allows for a rapid assessment of potential risk factors and an estimation of the risk associated with these factors.

In this case-control study, we investigated 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. Current tree and stand conditions were used as indicators of past conditions at the site. Cases consist of trees with *Phytophthora* cankers but generally not later symptoms of *Phytophthora*-related decline (i.e., *H. thouarsianum* fruiting or beetle attack). Controls are asymptomatic, though not necessarily uninfected trees. Cases and controls were sampled within areas where the disease syndrome is prevalent. This reduces the likelihood that controls simply represent trees that have not been exposed to *Phytophthora* inoculum, although this possibility cannot be ruled out for all controls.

We measured midday stem water potential (McCutchan and Shackel 1992) at the end of summer to assess levels of plant water stress in cases and controls. This factor can be considered an indicator of preexisting water stress levels if *Phytophthora* infection does not substantially affect water transport or tree water potential. *Phytophthora* cankers affect the bark but generally do not affect substantial amounts of xylem tissue (Garbelotto et al 2001), at least in trees in early stages of disease that we selected as cases. Therefore, we assumed that case trees were not likely to exhibit changes in stem water potential brought about by infection with *Phytophthora*.

We also collected data on a variety of tree, stand, and plot variables that might influence disease risk, including the presence of various other disease agents. Due to limitations of time and funding, we concentrated our efforts on one host species, coast live oak. For comparative purposes, we also collected a limited amount of data on tanoak.

METHODS

Study protocols

Study site selection

During September 2000, we established plots and collected data at 12 study locations (Table 1). Study sites were selected on the basis of appropriate vegetation type (adequate representation of host species 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 the disease syndrome is prevalent to minimize 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 and collected data on 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 stem water potential measurements and terrain. After determining that infected trees (cases) were present in adequate numbers in the stand, we selected a random starting point and searched for the nearest case or control tree. 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. For all locations except location 9, we used a GPS receiver to determine the coordinates of one or more plots at each location.

Stem water potential determinations

We collected midday stem water potential (stem Ψ) readings on the center subject tree in each plot during the 2 hour peak midday period (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) arising directly from 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 stem Ψ, but two readings were made on many trees as a check on the technique. In general, two valid stem Ψ measurements from a single tree were within 0.05 to 0.1 MPa of each other. Stem Ψ measurements were made with a pump-up pressure chamber (PMS Instrument Co., Corvallis OR) fitted with a 4 inch 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 stem Ψ readings were made, we recorded the minimum and maximum temperature and relative humidity values using a portable electronic thermohygrometer (Mannix TH Pen, model PTH8708). The thermohygrometer was placed in a ventilated shelter mounted on a mast and was positioned near the upper portion of the tree canopy layer during the observation period. VPD was calculated from the average of the recorded minimum and maximum temperature values using the following formula:

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

where:

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

Additional tree and plot variables

Other plot and tree variables we assessed are listed in Tables 2 and 3. A copy of the data sheet used in the field is included at the end of this report. Basal area was measured by the Bitterlich method using a variable radius plot. 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. *Phytophthora* girdling rank was derived by grouping percent girdling estimates into the following classes: 0 = 0%, 1 = 1% to 19%, 2 = 20% to 39%, 3 = 40% to 59%, 4 = >59%. 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 fitted logistic regression models to the data to examine the effects of factors on the binary disease outcome (subject tree is diseased, i.e., a case). The parameters obtained from this type of model are odds ratios. The odds ratio is the odds of an outcome given (x+1) divided by the odds of an outcome given x, i.e.:

$$\text{odds ratio} = \frac{\text{odds}(\text{case}|x+1)}{\text{odds}(\text{case}|x)} \qquad \text{(Equation 2)}$$

where x and x+1 are two successive levels of a factor. The odds that a tree will be diseased (a case) given a factor x is the probability of disease given x divided by the probability of no disease (i.e., tree is a control) given x. Mathematically this is expressed as follows:

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

If the odds ratio is **greater than one**, an outcome is **more likely** as levels of x increase. If the odds ratio is **less than one**, an outcome is **less likely** as levels of x increase. An odds ratio of one would indicate the levels of the factor being compared have no effect on the outcome variable. 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 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 stem Ψ. In this example, the odds ratio for n MPa difference in stem Ψ would be the per MPa odds ratio raised to the power n.

Table 2. Plot and stand variables measured in study plots. All variables except basal area were measured in the 8 m radius fixed area plots.

Variable	Method	Scale/units	Notes
Basal area	survey laser reticle	m^2/ha	reticle BAF = 5 m^2/ha
Tree density / species composition	count by species	count	multi-stemmed trees count as single trees; plot area = 0.02 ha (0.05 ac); trees have at least one stem at least 3 cm DBH
Slope	clinometer	percent	
Aspect	compass	degrees	
Tree canopy cover	visual estimate	pretransformed 0-6 scale*	
Phytophthora-related disease incidence	count by host spp. and symptom class	count	Symptom classes: 1 - asymptomatic 2 - early disease (bleeding cankers only) 3 - late disease (bleeding cankers, *Hypoxylon* &/or beetles) 4 - dead (with evidence of *Phytophthora* cankers)
Other decline and mortality	counts by host spp. of trees killed or in severe decline due to agents other than *Phytophthora*	count	only *Phytophthora* host tree species are scored; dead trees tallied only if estimated to have died within the past 10 years
Other pathogens/agents	note presence of various agents and symptoms on host species	present in plot	agents include various decay fungi, canker rot, *H. thouarsianum*, and beetles
Understory woody cover	visual estimate	pretransformed 0-6 scale*	includes woody regeneration in understory
Host sp. regeneration	count or estimate if >10	count	regeneration: <3 cm dbh
Phytophthora-related disease incidence in regeneration	count or estimate percent if count > 10	percent	
Disturbance	roads, trails, etc within plot or near edge of plot	present in plot	type of disturbance was noted

* The 0-6 scale is based on an 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. Tree variables measured for the subject tree in each study plot

Variable	Method	Scale/units	Notes
Subject tree species			*Q. agrifolia* or *L. densiflorus*
Origin class	visual assessment	seed or sprout	
Number of stems from ground	count	stems/tree	
DBH	estimated with flat tape	cm	
Midday stem water potential	pump-up pressure chamber	bars	readings made between about 1 and 3 pm after leaves had equilibrated at least 2 h in opaque reflective bags
Unshaded canopy percentage	visual	pretransformed 0-6 scale[1]	Percent of canopy projection area with unobstructed access to direct overhead sunlight
Crown class	visual	1-5 scale	Based on relative amount of shading: 1 - open grown 2 - dominant 3 - codominant 4 - intermediate 5 - overtopped
Phytophthora canker count	visual estimate	count	estimated based on external bleeding spots and limited inspection of canker margins
Percent girdling due to *Phytophthora* canker	visual estimate	percent of circumference	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
Canopy thinning	visual	0-2	Scale: 0-none, 1-slight, 2-pronounced
Epicormic branches	visual	0-2	Scale: 0-none, 1-few, 2-numerous
Canopy dieback	visual	pretransformed 0-6 scale[1]	Percent dead crown volume
Decay impact[2]	visual	0-3	Scale: 0-no , 1-low, 2-moderate, 3-high

[1]The 0-6 scale is based on an 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 Assessment of actual levels of decay in standing trees is problematic. The decay impact rating (Swiecki et al 1990) assesses the probability that existing decay will have a significant negative impact on tree health or survival. The 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.

We also used ordinal logistic regression (SAS 2000) to develop models for girdling rank, an ordinal (ordered categorical) disease severity outcome variable. *Phytophthora* girdling rank was derived by grouping percent girdling estimates into the following classes: 0 = 0%, 1 = 1% to 19%, 2 = 20% to 39%, 3 = 40% to 59%, 4 = >59%. For an ordinal response variable, the cumulative probability of being at or below each response level is modeled with logistic regression curves. The curves are the same for each level of the outcome variable except that they are shifted to the right or left. The ordinal logistic model fits a different intercept, but the same slope, for each of r–1 cumulative logistic comparisons, where r is the number of outcome response levels. For these analyses, odds ratios were not calculated and we report only the significance level of the likelihood ratio test for each factor in the model.

We screened possible predictor variables using univariate logistic regressions. 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. We developed multivariate models using a stepwise procedure. Factors were generally considered for entry into the multivariate models if odds ratios from univariate models were significant at $P \leq 0.10$. The reported significance level of each factor in a multivariate model is dependent upon the other factors which are included in the model. Therefore, the significance level of each factor reported in the final models should be interpreted as if it were the last factor added to the model.

Other linear models and tests

We used linear regression, analysis of variance, and analysis of covariance to test for associations between continuous outcomes (e.g., stem Ψ) 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.

RESULTS

We collected data at 12 locations on 150 subject trees (cases and controls). For coast live oak, we collected data on 75 controls (trees lacking *Phytophthora* symptoms) and 53 cases (trees displaying early *Phytophthora* symptoms). The relatively small tanoak sample included 9 cases and 13 controls. Overall, controls outnumber cases because trees showing early *Phytophthora* symptoms were often difficult to locate when following the sampling pattern that we employed for establishing plots.

Because it was difficult to find adequate numbers of trees displaying only *Phytophthora* cankers, 9 of the coast live oak case trees had minor amounts of beetle damage or a few small *H. thouarsianum* fruiting bodies, typically on a single scaffold or on a localized portion of the bole. We coded these trees as having more advanced disease symptoms so they could be handled separately in data analyses. Various analyses of subject tree variables failed to show any significant differences between these 9 trees and those with bleeding cankers only, so all cases have been aggregated in the analyses reported below.

Subject tree variables

Stem water potential

In early stages of disease, *Phytophthora* cankers affect the bark but generally do not affect substantial amounts of xylem tissue. Because we selected trees with early disease symptoms as cases in this study, we assumed that the presence of *Phytophthora* cankers would not affect midday stem water potential (stem Ψ) readings. Under this assumption, stem Ψ is an indicator of levels of plant water stress that the tree would have experienced irrespective of its disease status. A significant relationship between disease and stem Ψ could exist if high or low stem Ψ levels were either (1) a predisposing factor for disease development or (2) an indicator of other tree or plot conditions that favor disease.

Stem Ψ measurements varied widely among subject trees within locations (Figure 1). McCutchan and Shackel (1992) have shown that midday stem water potential is negatively correlated with vapor pressure deficit (VPD), which can be expressed as a function of temperature and humidity. Due to varying weather conditions over the 4-week period that we conducted our field work, VPD levels varied widely between different study locations (Figure 1).

The overall effect of VPD on stem Ψ can be illustrated by a comparison of stem Ψ readings taken on the same set of 7 coast live oak trees at location 1 on two days with widely different VPD levels (0.59 and 3.75 KPa). Average stem Ψ readings for these trees were significantly lower (indicating greater water stress) on the date with the higher VPD ($P=0.01$, matched pairs t-test). However, the decrease in stem Ψ was not consistent for all trees and ranged from 0.05 to 0.575 MPa. This suggests that factors other than VPD alone affect daily stem Ψ readings. We used analysis of covariance to determine which tree or plot factors were related to stem Ψ for both species, but only coast live oak had a sample size adequate to examine several factors in a single model.

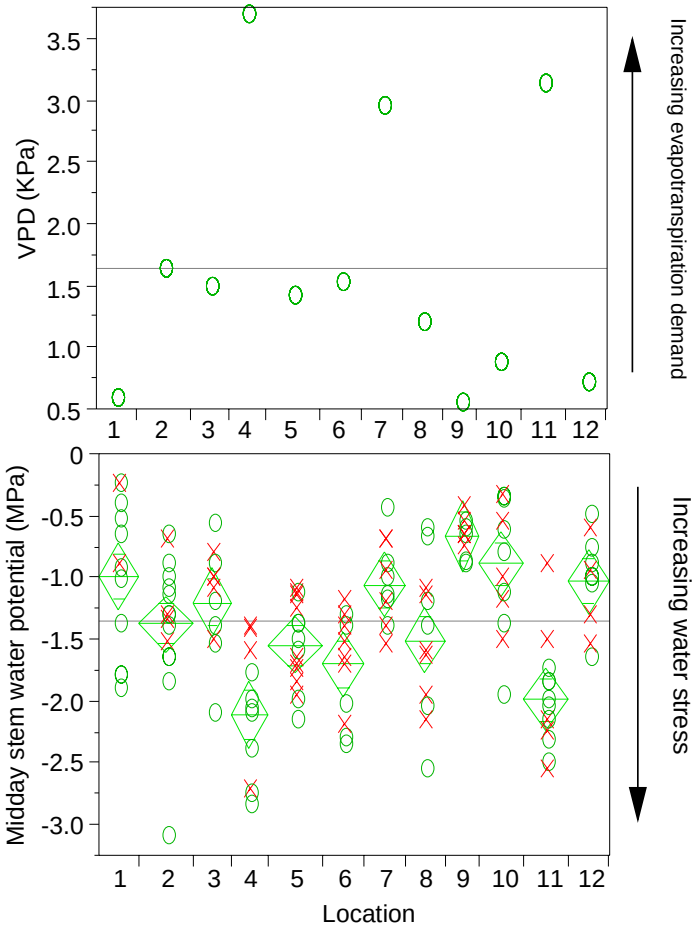

Figure 1. Top graph: Calculated average vapor pressure deficits (VPD) for each location during the period that stem water potentials were measured. Bottom graph: Stem water potentials for individual subject trees at each location (O=control trees, X=case trees). At locations 9 and 12, subject trees are tanoak; at all other locations subject trees are coast live oak. The horizontal line in each graph represents the overall average. The center line of each diamond represents the location mean; the vertical extent of each diamond represents the 95% confidence interval based on a pooled variance for all trees. The horizontal spread of each diamond is proportional to the sample size for each location.

Coast live oak. Our final analysis of covariance model for coast live oak accounted for about half of the variation in stem Ψ (Table 4). Consistent with the effect seen in the 7 trees described above, stem Ψ was negatively correlated with VPD overall. However, stem Ψ was also correlated with two variables related to the amount of sunlight that the tree canopy receives. Insolation was negatively correlated with stem Ψ, indicating that trees in plots that receive lower amounts of solar radiation (e.g., north-facing slopes) tend to have lower levels of water stress (i.e., higher water potentials). The amount of unshaded canopy (percent of the tree canopy that would be exposed to direct overhead sunlight) was also negatively correlated with stem Ψ, indicating that water stress was lower (stem Ψ was generally higher) in trees with heavily shaded canopies.

Table 4. Analysis of covariance model for midday stem water potential (MPa) of coast live oak

Source	DF	F Ratio	Prob>F	Adjusted R^2	n
Overall model	6	21.67	<0.0001	0.517	117
Model terms	**DF**	**F Ratio**	**Prob>F**	**Parameter estimate**	
Vapor pressure deficit (KPa)	1	5.06	0.0026	-0.101	
Unshaded canopy	1	37.45	<0.0001	-0.172	
Annual insolation (MJ/m^2)	1	10.33	0.0017	-0.000153	
Phytophthora girdling rank	1	7.59	0.0069	0.0930	
Decay impact rating	1	6.24	0.0140	0.120	
Interaction: Unshaded canopy >50% [true] × (VPD-mean VPD)	1	5.58	0.0199	-0.108	
Intercept				0.0920	

A significant negative correlation between stem Ψ and VPD only existed for trees with unshaded canopy ratings of 4 or higher (more than 50% unshaded canopy) (Figure 2). The model term describing the interaction between VPD and unshaded canopy >50% was significant in the final model (Table 4). In heavily shaded trees, low light levels may result in stomatal closure. This would reduce transpiration and uncouple the normal relationship between stem Ψ and VPD. Even if light levels were sufficient to maintain open stomata in leaves of overtopped branches, shaded leaves deep in the canopy may be exposed to a different VPD than what we measured at the top of the canopy. Regardless of the mechanism involved, our data indicate that canopy shading status must be taken into account when interpreting stem Ψ readings in coast live oak. Griffin (1973) noted a similar effect in his measurements of leaf Ψ in understory coast live oak saplings.

Stem Ψ was also correlated with two variables related to tree health (Table 4). Variables describing *Phytophthora* presence or severity (canker count, percent girdling, girdling rank, case/control) were positively correlated with stem Ψ. Girdling rank was the most highly significant of these variables and was included in the final model (Table 4). Stem Ψ was higher (indicating lower water stress) in cases than controls and the severity of *Phytophthora* girdling generally increased as stem Ψ increased. This result seems to confirm our assumption that early stages of *Phytophthora* infection seen in cases would not adversely affect stem Ψ. We infer that the positive association between disease and high stem Ψ indicates that trees located in relatively moist areas are at higher risk for disease than those located in drier sites.

Stem Ψ was also positively correlated with decay impact ratings in the subject tree (Table 4). We infer that this association is related to the likelihood that moist sites favor the development of wood decay through effects on the host and/or the wood decay fungi involved. However, other tree condition variables, including canopy thinning and dieback, were not correlated with stem Ψ. This suggests that stem Ψ is not elevated in diseased trees simply due to a reduction in the amount of transpiring leaf area.

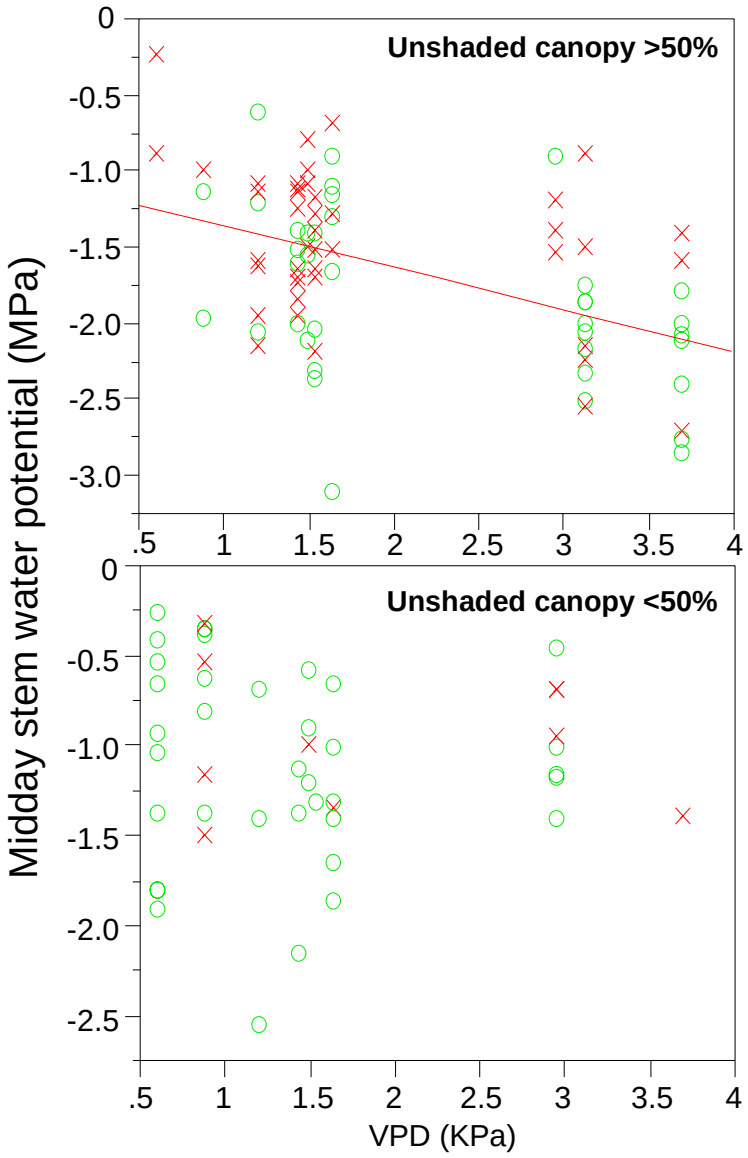

Figure 2. Relationship between midday stem water potential and vapor pressure deficit (VPD) for coast live oak trees with different levels of unshaded canopy (percent of the tree canopy exposed to direct overhead sunlight). Linear regression equation for unshaded canopy >50%: Midday stem Ψ (MPa) = -1.079 - 0.2742 VPD (KPa). Model adjusted R^2=0.21, P<0.0001, n=81.

Stem Ψ variables used in logistic regressions. Because stem Ψ readings were taken on different days, we needed to adjust the observed stem Ψ readings to account for the effect of VPD in order to create valid stem Ψ variables for use in logistic regression models. After testing a number of different adjustments, we found two variables that successfully removed the effect of VPD on stem Ψ. The first, difference from stem Ψ_{max}, is the difference between the measured stem Ψ and the maximum stem Ψ measured among all trees on that date. This variable was only marginally significant in a univariate logistic regression model for the binary disease outcome (case) (Table 5). Because difference from stem Ψ_{max} is expressed as a positive integer, it is negatively associated with the disease outcome, i.e., disease is less likely in trees that show increasingly greater levels of water stress. The second variable, adjusted stem Ψ, was more

highly significant than difference from stem Ψ_{max} in univariate logistic regression models for the binary disease outcome (Table 5). We calculated adjusted stem Ψ for trees with unshaded canopy ratings of 4 or higher using the slope from a simple linear regression model for VPD and stem Ψ (-0.2742 MPa Ψ/KPa VPD), which was developed only from this subset of trees (Figure 2, top).

Tanoak. For tanoak, VPD was the best predictor for midday stem Ψ in the analysis of covariance model. The small sample size limited our ability to fit other predictors in the model. As seen in coast live oak, average stem Ψ was negatively correlated with VPD. We also detected a significant negative correlation between unshaded canopy rating and stem Ψ in tanoaks. However, only 4 trees had unshaded canopy ratings as high as 4 (50-80%), so further observations are needed to confirm this relationship. The average stem Ψ of control tanoaks was about 0.05 MPa less than that of the cases, which is not a significant difference. No disease variables were correlated with stem Ψ in this small sample and stem Ψ variables were not significant predictors of disease in logistic regression models for tanoak.

Table 5. Odds ratios and 95% confidence intervals for predictors of *Phytophthora* infection (binary outcome) in univariate logistic regression models for coast live oak.

Predictor variables	Effect direction	Likelihood ratio Prob > χ^2	Odds ratio	Odds ratio 95% confidence interval
Subject tree variables				
Difference from stem Ψ_{max} (MPa)	-	0.0533	0.17	0.026 - 1.0
Adjusted stem Ψ (MPa)	+	0.0427	7.5	1.1 - 57
Canopy dieback rating[1]	+	0.0410	5.3	1.1 - 28
Canopy dieback >20%	+	0.0907	1.9	0.91 - 3.8
Unshaded canopy rating	+	0.0012	11	2.5 - 57
Unshaded canopy >50% [true]	+	0.0003	4.2	1.9 - 9.9
Crown class	-	0.0034	0.088	0.014 - 0.46
∑ DBH (cm)	+	0.018[2]	17	1.6 - 240
Number of stems	+	0.0061	24	2.4 - 330
More than 2 stems [true]	+	0.0007	9.6	2.4 - 64
Plot variables				
California bay count	+	0.0024[3]	46	3.4 - 1200
Phytophthora present in plot[4]	+	0.0127	2.5	1.2 - 5.2
Count of all *Phytophthora*-infected trees[4]	+	0.0254	6.5	1.3 - 40
Count of trees with early *Phytophthora* symptoms[4]	+	0.0145	8.1	1.5 - 52
H. thouarsianum fruiting present	+	0.0021	3.1	1.5 - 6.5
Beetle damage present	+	0.0002	4.1	2.0 - 9.0

[1] Ratings of 0 and 1 were pooled due to low sample sizes in these classes.
[2] Significance level drops to 0.0877 (OR=3.9 [0.82-19]) if 2 extreme values are excluded.
[3] Significance levels drop to 0.0393 (OR=5.5 [1.1-31]) if 3 extreme values are excluded.
[4] Predictor variables exclude infection of the subject tree.

Tree disease symptoms

Even though we selected case trees that had disease symptoms that characterize the early phase of the disease syndrome, disease severity varied substantially among cases. Estimated canker counts ranged from 1 to 22 (median 7) and estimates of bole girdling ranged from 1% to 90%

(median 20%). Frequency distributions for both symptom types were non-normal and were noticeably left-skewed (i.e., low values were more common than higher values).

Cases had significantly greater canopy dieback ratings than controls for both coast live oak and tanoak (F test P=0.038 and 0.009, respectively). Dieback rating was also a significant predictor of disease in logistic regression models for both coast live oak (Table 5) and tanoak. To test whether elevated dieback ratings could be the result of *Phytophthora* infection, we also constructed ordinal logistic models for girdling rank using only live oak cases (i.e., trees with girding ranks >0). Among the cases, canopy dieback was not correlated with the severity of *Phytophthora* symptoms (canker count, girdling percentage, or girdling rank). This result suggests that the canopy dieback we observed in these trees was not the result of *Phytophthora* infection. This agrees with our field observation that the pattern of canopy dieback in cases was not typical of that associated with *Phytophthora*-related decline. High levels of dieback due to other factors, such as twig canker or canker rot fungi, may be a predisposing or aggravating factor for *Phytophthora* infection.

Mean levels of other subject tree condition variables, i.e., canopy thinning, decay impact, and the abundance of epicormic branches, did not differ significantly between coast live oak cases and controls. However, canopy dieback, thinning, and epicormic ratings were positively correlated with decay impact ratings in coast live oak. These symptoms are often interrelated and occur in trees declining due to infections by wood decay fungi. For tanoak, decay impact was marginally greater in controls than in cases (P=0.0597, F test).

We did not see obvious evidence of *Phytophthora* symptoms in coast live oak regeneration. Possible *Phytophthora* symptoms were seen in tanoak regeneration in 8 of the 35 plots (23%) in which tanoak regeneration was tallied. However, dieback symptoms seen in tanoak regeneration are not diagnostic and could be due to other agents. We did not attempt to assay for the presence of *Phytophthora* in symptomatic seedlings. The average densities of coast live oak and tanoak regeneration did not differ between case and control plots. Among the 128 plots containing coast live oak trees, 50 (39%) lacked any live oak regeneration. In contrast, only 2 (7.7%) of the 26 plots containing tanoak trees lacked tanoak regeneration.

Other tree variables

Canopy shading. Levels of canopy shading were significantly greater in live oak controls compared to cases (P=0.0015, F test). Both unshaded canopy rating and a binary variable based on this factor (unshaded canopy >50%) were significant predictors of disease for this species (Table 5). Crown class, which is highly correlated with unshaded canopy, was also a significant predictor of disease (Table 5). Univariate logistic regression models indicate that live oak trees with lower levels of canopy shading (i.e., higher or more dominant canopy classes) were more likely to be diseased than were more heavily shaded trees.

As noted above, disease is also associated with higher stem Ψ levels, which in turn are associated with higher levels of canopy shade. Among live oaks with more than 50% unshaded canopy, all stem Ψ variables (stem Ψ, adjusted stem Ψ, difference from stem Ψ_{max}) indicate that cases have significantly higher Ψ (lower water stress) than control trees (all $P<0.05$, t-tests). Thus, it appears that among trees with more than 50% unshaded canopy, disease is more likely to occur in trees with high stem Ψ. However, none of these stem Ψ variables differ between cases and controls for trees with less than 50% unshaded canopy, so factors other than stem Ψ may have more influence on disease risk in trees that are more heavily shaded.

One possible explanation for the association between exposed canopy and disease could be that trees with greater canopy exposure may intercept airborne inoculum produced outside of the plot more readily than overtopped trees. Intercepted inoculum might then be channeled to the lower trunk via water that flows along stems during a rain event. Further investigation of canopy shading will be needed to clarify its relationship with disease risk in live oak.

Unshaded canopy rating and crown class were not significant predictors of disease in tanoak.

Main stem size. We examined several variables related to trunk diameter and/or trunk surface area, including the DBH and cross-sectional area of largest stem and of all main stems. Only the sum of all main stem diameters (Σ DBH), which is directly proportional to main stem circumference and surface area, was significant in logistic regression models for the binary disease outcome. However, the significance of this variable was greatly reduced if two extremely high Σ DBH values are omitted from the analysis (Table 5), which suggests that this variable is probably not associated with disease risk. None of the stem size variables were significant predictors of disease for tanoak.

Main stem count and sprout origin. For live oak, the number of main stems in the subject tree (which ranged from 1 to 6) was also a predictor of the binary disease outcome (Table 5). Although cases were more likely than controls to have more than two stems (Table 5), a binary variable indicating that a tree was multistemmed (more than 1 stem) was not significant. It is possible that at least some of the association between multiple stems and disease is due to the fact that a tree with multiple stems has more chances that at least one stem will have a *Phytophthora* canker. Because we did not rate disease in each main stem individually, we cannot directly determine whether multiple stems confer any elevated disease risk beyond this statistical effect.

The sprout origin rating was associated with the number of main stems. Only trees rated as being of sprout (coppice) origin had more than 2 stems. The percentage of sprout origin trees was higher among live oak cases (53%) than controls (36%). However, sprout origin rating was only significant at P=0.058 (likelihood ratio test) in a univariate logistic model for the binary disease outcome.

Neither stem count nor sprout origin variables were significant in tanoak disease models. Overall, 45% of the tanoak subject trees were multistemmed and 59% were scored as sprout origin. By comparison, 65% of coast live oak subject trees were multistemmed and 43% were scored as sprout origin.

Plot variables

Stand density and composition

In addition to the data collected on the subject trees, we also rated disease on a total of 737 coast live oak, California black oak, and tanoak trees that were located within the 150 plots. Multiple species of these three known *Phytophthora* hosts occurred only rarely within study plots. The two subject hosts of this study, coast live oak and tanoak, occurred together in only four plots; these were all at location 3 (Table 6). California black oak occurred in plots at low frequencies in six of the 12 locations where live oak was the subject tree. Other oaks occurred uncommonly in study plots. Valley oak (*Q. lobata*, a white oak) was present in a total of 18 plots at 7 live oak locations (1,4,5,6,8,10,11). Canyon live oak (*Q. chrysolepis*, an intermediate oak) was present in a single plot at location 12.

California bay (*Umbellularia californica*) occurred at all locations and was present in 61% of all plots. The next most common tree species in plots were madrone (*Arbutus menziesii*) and Douglas fir (*Pseudotsuga menziesii*) in 36% and 27% of the study plots, respectively. Coast redwood (*Sequoia sempervirens*) was found only within tanoak plots, although it was present within 100-200 m of study plots at live oak locations 3, 7, and 10. Other tree species occurring infrequently in plots included California buckeye (*Aesculus californica*) (live oak locations only), bigleaf maple (*Acer macrophyllum*), hazel (*Corylus cornuta* var. *californica*), and California nutmeg (*Torryea californica*) (one tanoak location only). Average tree densities and stand composition by species for the study locations are shown in Table 6.

Total tree density, host tree density, and basal area within plots varied significantly between locations ($P<0.01$, F test). However, none of these variables differed significantly between case and control plots, and these variables were not significant predictors of disease risk in the logistic regression models. Of these variables, only plot basal area (exclusive of the subject tree) differed between tanoak and live oak plots. Basal area was significantly greater in tanoak plots (mean 65 m^2/ha) than live oak plots (mean 35 m^2/ha), due to the presence of large-diameter redwood and Douglas fir in the tanoak locations (9 and 12).

Although overall and host tree density were not related to the presence of disease, densities of certain trees that are not currently known to be hosts of *Phytophthora* sp. nov. were associated with disease. For live oak, the density of California bay within the plot was a significant positive predictor of disease (Table 5). However, three case plots with high bay densities (1400 to 1600 trees/ha) had a large influence on this association. The significance level of the effect of California bay density is greatly reduced if these three plots are excluded from the analysis, although the effect is still significant. Densities of other species, including those of the individual host species, were not related to disease for live oak.

At least two related phenomena could explain the positive relationship between bay density and disease. It may be that within coast live oak woodlands, California bay is an indicator of conditions that are more favorable for disease development, such as moist sites. Although the ranges of California bay and coast live oak overlap substantially, bay tends to be restricted to more mesic areas or areas with higher levels of soil moisture, such as drainages (Griffin and Critchfield 1976). Second, because bay produces relatively dense evergreen shade, the presence of high bay populations might help create a favorable microclimate for disease development, for instance by slowing the rate at which stems dry. As additional information about the biology of *Phytophthora* sp. nov. becomes available, these and other possible explanations can be evaluated in more detail.

For tanoak, the low sample size severely restricts the power of the analysis. Redwood density showed a significant negative association with disease for tanoak, but more extensive sampling would be needed to determine whether this relationship holds for larger populations.

Table 6. Stand density, basal area, and stand composition for the most common trees by location. Subject host trees at locations 9 and 12 (bold italics) are tanoak; live oak is the subject host at the remaining locations. Dead host trees are included in these figures.

Location	Total[1] trees/ha	Basal area m^2/ha	Percent of trees within plots						
			Tanoak	Live oak	Black oak	California bay	Madrone	Coast redwood	Douglas fir
1	746	34.0	0%	36%	0%	15%	16%	0%	28%
2	597	37.2	6%	60%	0%	10%	11%	0%	18%
3	523	35.9	0%	63%	4%	24%	2%	0%	3%
4	527	22.5	0%	46%	0%	32%	2%	0%	0%
5	841	25.3	0%	18%	1%	16%	55%	0%	0%
6	1021	26.8	0%	20%	1%	34%	34%	0%	0%
7	1067	45.0	0%	31%	2%	43%	23%	0%	1%
8	412	47.9	0%	63%	0%	34%	0%	0%	0%
9	***695***	***79.0***	***67%***	***0%***	***0%***	***4%***	***0%***	***24%***	***1%***
10	662	43.5	0%	28%	1%	17%	12%	0%	49%
11	400	34.2	0%	68%	1%	16%	0%	0%	0%
12	***588***	***54.2***	***45%***	***0%***	***0%***	***14%***	***1%***	***30%***	***4%***

[1] Density includes subject tree in plot.

Disease levels within plots

Phytophthora. Using data on disease symptoms for the three *Phytophthora* host species in plots, we created a number of related variables to analyze the relationships between disease levels in the plot (exclusive of the subject tree) and disease in the subject tree. Host tree densities were similar in case and control plots. However, *Phytophthora*-infected trees occurred in significantly greater numbers in case plots than in control plots (Figure 4). We performed a two-way analysis of variance on percent *Phytophthora* infection of plot trees (arcsine transformed data) using plot type (case/control) and subject tree species as main effects. In this analysis, plot type was significant at P=0.01, species was significant at P=0.058, and the interaction term was not significant.

Overall, the percentage of host trees with *Phytophthora* symptoms was somewhat higher in tanoak plots (53% in cases, 27% in controls) than in live oak plots (33% in cases, 18% in controls). The same pattern is seen for the incidence of plots with *Phytophthora* symptoms in trees other than the subject tree. For tanoak, 89% of case plots and 69% of control plots had at least one tree with *Phytophthora* symptoms (excluding the subject tree). For live oak, 62% of case plots and 40% of control plots had at least one tree with *Phytophthora* symptoms (excluding the subject tree). Current levels of *Phytophthora*-related mortality among all host trees were also much higher in tanoak plots (22.2% in cases, 13.6% in controls) than in live oak plots (6.6% in cases, 3.2% in controls). Table 7 presents the incidence of different *Phytophthora* symptoms by location and plot type for all 150 plots. Table 8 presents the incidence of different *Phytophthora* symptoms by host species and plot type for all 150 plots.

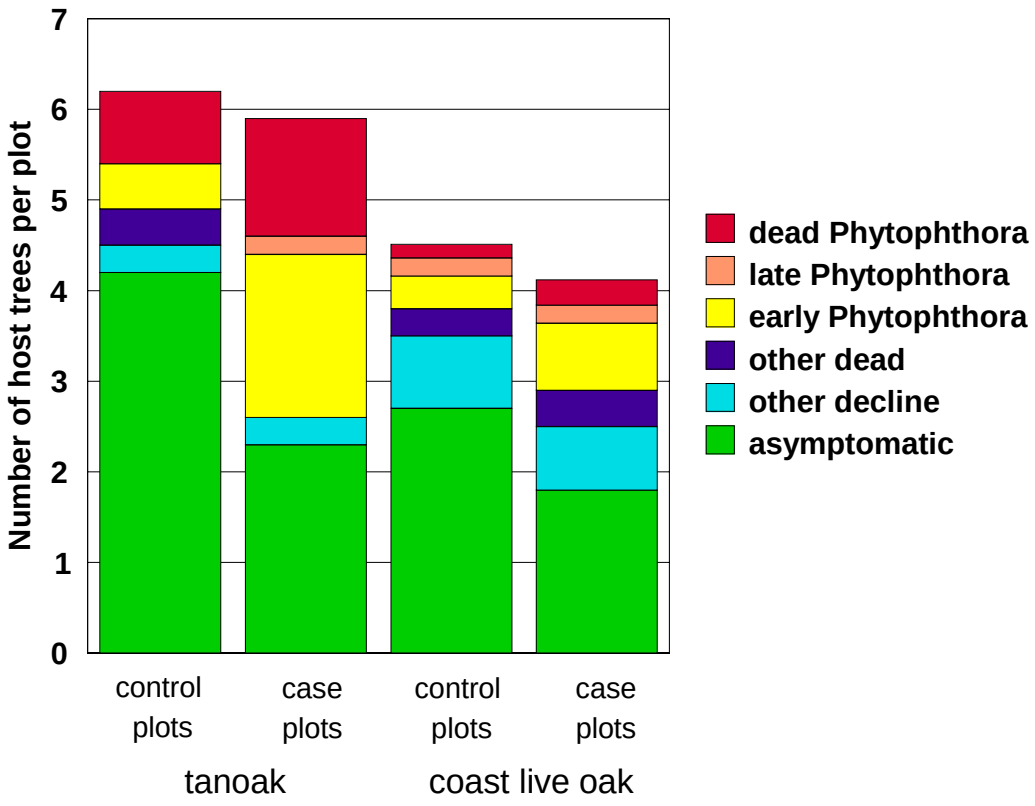

Figure 4. Average number of host trees per plot showing symptoms or mortality related to *Phytophthora* infection or other disease agents for tanoak and coast live oak case and control plots. The case or control subject tree from each plot is not included in the percentages. Dead trees were counted only if mortality was estimated to have occurred within the past 10 years.

Several variables related to *Phytophthora* symptoms in plot trees were significant predictors of disease in the subject tree for both live oak (Table 5) and tanoak (not shown). These analyses indicate that for both species the risk of disease in a subject tree is elevated if other infected trees are present in the plot. From this, we infer that infected trees are spatially aggregated, at least on the scale of the plot size used in this study (0.02 ha).

For live oak, the risk that a subject tree will have early disease symptoms increases with the number of other host trees in the plot showing early *Phytophthora* symptoms, i.e., bleeding but no evidence of beetle attack or *H. thouarsianum* fruiting (Table 5). However, the number of plot trees with late *Phytophthora* symptoms and/or dead trees with *Phytophthora* symptoms were not significant predictors of disease. Furthermore, the number of plot trees showing late disease symptoms was not correlated with the number of plot trees showing early disease symptoms. For tanoak a similar relationship exists; only the total count of trees with *Phytophthora* symptoms and the count of trees with early *Phytophthora* symptoms were significant predictors of disease. However, the sample size for tanoak is too small to provide a high level of confidence in the relationship for this species.

Table 7. Percent of Phytophthora host trees (tanoak, coast live oak, and black oak) in each disease class by plot type (case or control) at each location. Data exclude the plot subject tree. The subject tree species at locations 9 and 12 was tanoak. At all other locations the subject tree species was coast live oak.

Location	Plot type	Number of host trees	% early *Phytophthora*	% late *Phytophthora*	% dead *Phytophthora*	% non-*Phytophthora* decline	% non-*Phytophthora* dead
1	control	44	14%	0%	0%	41%	2%
	case	9	22%	11%	0%	11%	0%
2	control	74	4%	4%	3%	12%	1%
	case	26	4%	0%	0%	8%	4%
3	control	39	5%	8%	13%	18%	8%
	case	27	30%	4%	7%	4%	19%
4	control	27	7%	0%	0%	19%	0%
	case	15	20%	0%	0%	33%	13%
5	control	22	9%	5%	0%	14%	0%
	case	15	33%	7%	0%	33%	7%
6	control	9	11%	0%	11%	11%	33%
	case	30	30%	7%	23%	10%	7%
7	control	39	10%	0%	3%	15%	5%
	case	34	6%	6%	12%	21%	12%
8	control	19	5%	26%	5%	5%	21%
	case	31	13%	10%	3%	26%	6%
9	control	44	11%	0%	5%	7%	9%
	case	39	38%	5%	8%	5%	0%
10	control	27	7%	0%	0%	15%	26%
	case	10	40%	10%	0%	10%	0%
11	control	40	10%	10%	3%	10%	3%
	case	19	5%	5%	5%	16%	11%
12	control	37	3%	0%	24%	3%	3%
	case	15	7%	0%	60%	7%	0%

Table 8. Incidence of symptoms associated with *Phytophthora*-related decline and mortality in study plots. Totals exclude symptoms on the plot subject tree.

Species	Plot type	Number of trees	Early symptoms	Late symptoms	Dead
Coast live oak	Control	321	7.2%	4.0%	2.8%
	Case	251	14.7%	4.8%	6.0%
California black oak	Control	7	14.3%	14.3%	0%
	Case	11	18.2%	0%	0%
Tanoak	Control	93	9.7%	2.2%	14.0%
	Case	54	29.6%	3.7%	22.2%

If local inoculum (i.e., from trees within a 8 m radius) had played a major role in disease development in these plots, we might expect that late disease symptoms would be associated with early disease symptoms in the subject tree or other plot trees. While our data do not provide evidence for local disease cycling, we cannot fully interpret the results without knowing what

model of disease progress applies. In order to hypothesize what events or plot conditions would be consistent with the observed disease pattern, we need to know whether symptom development proceeds at similar or widely different rates among trees in a given plot. Irrespective of the disease progress model that applies, our data suggest that on a local scale (0.02 ha) spatial aggregation of trees with early symptom development is not clearly related to the presence of more advanced disease symptoms within the plot.

Other diseases and insects. Host tree decline and death associated with agents other than *Phytophthora* did not differ significantly (F test) between case and control plots for live oak or tanoak (Figure 4). For coast live oak, the number of trees affected by decline and recent mortality (estimated to have occurred within the past 10 years) associated with other agents (Table 9) was similar to the total percentage of affected by *Phytophthora* in case plots (Table 8) (28% and 25.5%, respectively). For tanoak, however, the percentage of trees affected by *Phytophthora* in case plots (56%, Table 8) is far in excess of the "background" decline and mortality associated with other agents (8%, Table 9).

Table 9. Host trees affected by severe decline or mortality associated with agents other than *Phytophthora* sp. nov. in study plots. Totals exclude symptoms on the plot subject tree.

Species	Plot type	Number of trees	Severe decline	Recent (10 yr) mortality
Coast live oak	control	321	17.4%	6.9%
	case	251	27.1%	6.0%
	all	**572**	**21.7%**	**6.5%**
California black oak	control	7	28.6%	0%
	case	11	27.3%	36.4%
	all	**18**	**27.8%**	**22.2%**
Tanoak	control	93	4.3%	5.4%
	case	54	5.6%	0%
	all	**147**	**4.8%**	**3.4%**

Evidence of bark and/or ambrosia beetle damage and fruiting bodies (stromata) of *H. thouarsianum* were relatively common on host trees in the plots (Table 10). For live oak, the presence of beetle damage or *H. thouarsianum* fruiting within the plot were significant predictors of the disease in the subject tree (Table 5). These predictors are associated with each other, with the number of *Phytophthora*-infected trees in the plot, and with the number of declining and dead host trees due to causes other than *Phytophthora*. It is probably more useful to view *H. thouarsianum* and beetle presence as outcomes related to *Phytophthora* canker rather than as independent predictors of disease.

No other disease or damage agents that we recorded were associated with the *Phytophthora* disease outcome. Of the other agents that are associated with oak mortality, canker rots (typically caused by *Inonotus* spp.) were the most common. Canker rot symptoms were far more likely to be found in live oak plots than in tanoak plots (Table 10). Many of the specific wood decay fungi were observed in a relatively small percentage of the plots (Table 10). However, because identification was based on the presence of identifiable fruiting bodies in September, the observed incidences are likely to greatly underestimate the actual incidences.

Table 10. Percent of plots with visible evidence of canker rot, beetle damage, or fungal fruiting bodies on *Phytophthora* host trees

Symptom type or agent	Coast live oak plots	Tanoak plots
canker rot symptoms without fruiting bodies	63.3%	14%
oak bark and/or ambrosia beetles	62.5%	36.4%
Hypoxylon thouarsianum	50.0%	31.8%
Phellinus spp.	10.9%	--
Inonotus andersonii	7.8%	5.0%
Other *Inonotus* spp.	0.8%	--
Ganoderma spp.	1.6%	--
Laetiporus sulphureus	0.8%	--

Particularly for live oak, we observed that *Phytophthora* symptoms sometimes occurred in trees that were declining due to canker rot infection or other agents that are not normally associated with *Phytophthora*-related mortality. Dead trees that had any evidence of substantial *Phytophthora* cankers were rated as *Phytophthora*-related mortality, even though some of these trees might have been dead at the time of the survey due to other diseases even if they hadn't been infected by *Phytophthora.* The inclusion of these trees as *Phytophthora*-related mortality may exaggerate the current impacts of *Phytophthora* to a minor degree.

Other plot variables

Other measured plot variables were not significantly related to disease in the subject tree in univariate models. These included total plot canopy cover, woody understory cover, plot slope, insolation (which integrates the effects of plot slope and aspect), or the presence of regeneration of various host or nonhost species in the understory.

Multivariate models

Many of the individual predictor variables discussed above (Table 5) are correlated or interrelated in various ways. By constructing multivariate logistic regression models, we can gain insight into the relative strength of various predictor variables and the degree to which predictor variables can be substituted for each other. In general, highly collinear variables cannot be fitted into the same model. Given several related and highly correlated variables, we selected the variable that improved overall model fit the best. Variables in the model can influence which additional variables are included. For example, although adjusted stem Ψ was a better predictor in univariate models, difference from stem Ψ_{max} generally provided a better fit in multivariate logistic regression models.

Given the limited size of our data set, the multivariate models should not be considered as absolute predictive models. The primary utility of the models is to identify individual variables or clusters of variables that appear to be related to the disease outcomes. Variables that appear in these models may warrant further investigation in studies of *Phytophthora* canker epidemiology and management. Inclusion of a factor in a model does not necessarily imply a causal relationship between the factor and the outcome. As noted above, predictor variables included in a model may in fact be outcomes that are influenced by the same underlying (and possibly unmeasured) factors that influence disease risk.

Coast live oak

For the binary outcome (case), we first fitted a multivariate model that included all significant predictors. Two predictors in this model, cross-sectional area of the largest stem and count of California bay in plot, had a few extremely high outliers in their data distributions. When we omitted these outliers from the analysis, these variables were no longer significant. We excluded these two predictors from the final model (Table 11), but used all available data points (n=127 due to missing data for one variable).

Overall, the final model correctly classified 77% of the subject trees as cases or controls, using P(outcome) > 0.5 as the criterion for predicting either outcome (case or control). The final model was more successful at predicting controls (87% correctly classified) than cases (65% correctly classified). Several factors may explain the tendency of the model to underpredict the case outcome. Most importantly, the model does not include variables related to factors that may be important in disease development, such as the genetic resistance level of individual trees. In addition, the data set used to develop the model may include a number of control trees that are infected but had not yet developed visible symptoms. If trees were reassessed in a follow-up survey, information about the change in disease status of control trees might improve model fit.

The magnitudes of the effects in the model are quite substantial, although the confidence intervals for the odds ratios are large due to the limited sample size. We expect that the model in Table 11 would not necessarily provide robust predictions of disease outcomes in stands other than those sampled. Nonetheless, it is likely that models developed for other stands would include one or more of the predictor variables in our final model. Therefore, these factors or closely related factors should be assessed in studies that attempt to model *Phytophthora* disease risk in coast live oak.

Table 11. Multivariate logistic regression model[1] parameter estimates for the binary disease outcome (case) for coast live oak.

Predictor variables	Likelihood Ratio Prob>χ^2	Odds ratio	Odds ratio 95% confidence interval
More than 2 stems [true]	0.0009	14.6	2.77 - 124
Unshaded canopy >50% [true]	<0.0001	10.6	3.67 - 36.0
Difference from stem Ψ_{max} (MPa)	0.0057	0.0430	0.00347 - 0.415
Canopy dieback >20% [true]	0.0062	3.66	1.43 - 10.2
Count of trees with early *Phytophthora* symptoms	0.0239	8.96	1.34 - 65.8

[1]Overall model likelihood ratio P<0.0001, n=127 (one record omitted due to missing data for one variable).

We also developed ordinal multivariate logistic models for the girdling rank outcome to determine whether disease severity was associated with any of the factors we measured. We again found that California bay count was fitted into the model only if the 3 high outliers were included, so we dropped this predictor from the model but continued to fit the model using all data points (n=126 due to missing data). Fewer parameters could be fitted into the girdling rank model (Table 12) than the final binary outcome model (Table 11). Also, none of the variables related to *Phytophthora* infected trees in the plot were significant in the girdling rank model, although the associated binary variable indicating the presence of beetle damage in the plot was significant. The fit of the multivariate ordinal model was much poorer than that of the binary outcome model. The model correctly assigned only 50% of the subject trees to the correct girdling class (calculated by rounding predicted values to the nearest whole rank). The ordinal

model tended to both overpredict girdling severity in controls (rating 0) and to underpredict girdling in the highest girdling classes (ratings 3 and 4).

To see whether poor fit of the ordinal model was related to the preponderance of controls (all with girdling rank 0) in the data set, we also constructed an ordinal model using only the cases (n=52). The fit of the case-only model (44% of girdling ranks among cases correctly predicted) was not as good as the case+control model. Although somewhat poorer fit was expected due to the reduced sample size, the case-only model showed the same tendency to underpredict the two highest girdling classes. Only two predictors could be fitted in the case only model (Table 12), with adjusted stem Ψ being the more significant variable.

Girdling rank is likely to be affected by rates of canker expansion and the presence of multiple successful infections on a tree. Girdling rank may not be an optimal outcome variable because trees could develop similar girdling ranks through either a few widely spreading cankers or a multitude of limited cankers. If different sets of factors affect canker initiation and canker expansion, it could be difficult to develop reasonable models using the girdling rank outcome, especially in a relatively small sample. Furthermore, genetic factors that influence host resistance could exert an even stronger influence on girdling rank than on disease occurrence. Because the tree and plot variables we measured do not reflect this genetic component, they may not be good predictors of girdling severity even if they have some value in predicting disease occurrence. Finally, the design of this case-control study was not optimized for assessing factors associated with disease severity. A cross-sectional study that includes more trees in the high disease severity classes may be able to detect more predictors of disease severity.

In multivariate models, subject tree variables were generally better predictors of disease than plot variables such as tree density and insolation. Several of the subject tree variables, including unshaded canopy, stem Ψ, and even stem cross-sectional area, are clearly related to characteristics of the plot. Nonetheless, individual tree characteristics may be more important determinants of disease risk than the plot characteristics that we rated.

Table 12. Significance levels (effect likelihood ratio χ^2 test) for ordinal logistic regression model parameters for the girdling severity rating outcome. Models were developed for all coast live oaks (cases+controls) or live oak cases only.

Predictor variables	Likelihood Ratio Prob>χ^2	
	Cases+controls	Cases only
Unshaded canopy >50% [true]	<0.0001	--
Difference from stem Ψ_{max} (MPa)	0.0038	--
Adjusted stem Ψ (MPa)	--	0.0094
Canopy dieback >20%	0.0102	--
Beetle damage in plot	0.0002	--
Canker rot present in plot	--	0.0609
n	126	52
Overall model	<0.0001	0.0071

Tanoak

The best fitting model for this species (Table 13) correctly classified 86.4% of the subject trees (7 of 9 cases and 12 of 13 controls). The significant negative relationship between *Phytophthora* symptoms and decay impact is probably largely related to the situation at location 12, where the largest plot trees were typically affected by canker rot but were lacking in *Phytophthora* symptoms. The negative relationship between insolation and disease suggests that trees in more

mesic sites (e.g., north facing slopes) were more likely to be diseased. However, given the small data set for this species, we do not know whether such relationships are likely to exist in areas beyond the two study sites.

Other than the canopy dieback variable, the tanoak model does not include variables that are related to those in the coast live oak model. Tanoak is apparently more susceptible than live oak to *Phytophthora* sp. nov., and typically occurs in moister and often shadier sites than coast live oak. It is therefore possible that factors influencing disease risk in the two species differ to some degree. Additional studies involving both species will be needed to explore these differences further.

Table 13. Multivariate logistic regression model parameter estimates for the binary disease outcome (case) for tanoak.

Predictor variables	Likelihood Ratio Prob>χ^2	Odds ratio	Odds ratio 95% confidence interval
Canopy dieback rating	0.0006	9107	20.6 - 1.26×10^9
Decay impact rating	0.0068	0.002672	5.8×10^{-7} - 0.26
Annual insolation (MJ/m^2)	0.0184	0.0005201	5.1×10^{-9} - 0.34

DISCUSSION

The primary objective of this study was to examine the relationship between water stress and *Phytophthora* infection. We found a significant positive association between disease and high stem Ψ, which does not support our original hypothesis that disease might be more common or severe in water-stressed trees. Instead, the data are consistent with the hypothesis that trees located in relatively moist areas are at higher risk for disease than those located in drier sites.

One or more of several possible explanations could explain why disease risk may be elevated for trees in moist sites. Environmental conditions associated with these sites could be especially favorable for the pathogen. For example, prolonged dew periods or extended periods of soil saturation might occur in these areas and could favor sporangium production, zoospore motility and germination, and/or infection. High levels of soil moisture could also influence host susceptibility. For example, prolonged periods of soil saturation could expose roots to hypoxic stress which can increase host susceptibility to *Phytophthora* infection (Sinclair et al 1987). Alternatively, high soil moisture could result in more rapid trunk growth, possibly increasing the amount of growth cracks in the bark or otherwise rendering the bark more susceptible to infection. Further investigations into the role of plant water status and moisture levels on plant surfaces and in soils will be needed to determine whether these factors could provide a means of predicting disease spread.

An inherent problem with correlative ecological studies is that many of the variables that can be measured are interrelated. For example, stem Ψ is correlated with unshaded canopy, insolation, and other variables (e.g., California bay count). Although stem Ψ and several of these other variables are related to disease risk, it is not clear which variables may be causally related to disease, which are related outcomes, and which may simply be spuriously correlated with disease. While causal relationships are of the greatest interest for determining disease epidemiology, related outcomes may be of use in modeling disease risk across the landscape. For instance, although stem Ψ may be more directly related to processes that directly influence

disease risk, it is relatively difficult and time consuming to measure. Easily observed predictor variables that may serve as indicators of conditions that favor disease risk are therefore of potential use for monitoring programs. For example, if variables related to stem Ψ and the presence of *Phytophthora* in the plot are removed from the final logistic model presented in Table 11, California bay count is significant (with outliers excluded) and would be fitted into the model. Hence, this variable could be used in place of variables that are more difficult to measure to predict disease outcome in our data set. Further investigation would be needed to determine if this or other variables would be useful indicators of disease risk in affected areas.

Relatively few predictors related to plot conditions were significant in our models. In part, this may be due to our relatively limited sample size, which only allows us to detect relatively large effects. Furthermore, we selected areas for study where *Phytophthora* was already common. Within these areas, it may be that *Phytophthora* canker is not overly limited by environmental conditions, or that the variation in critical environmental conditions across the locations was relatively small. Within these areas, tree-related variables may be relatively more important in determining disease risk. Hence, our models should not be interpreted to suggest that site factors do not strongly affect disease risk. Across the range of the host species, we believe that it is likely that environmental factors pose major constraints to disease development. Nonetheless, on a local scale, especially within an area that is favorable for disease development overall, tree-related factors may be the best predictors of disease risk.

Areas sampled in this study were limited to those where *Phytophthora* canker was common. Therefore disease levels in these plots may be greater than would be expected overall across affected areas. Furthermore, because plots were selected based on the occurrence of a case or control tree and were not random, they do not provide unbiased estimates of disease levels in the sampled stands. Nonetheless, the relative levels of disease and mortality associated with *Phytophthora* and other agents are worth noting. In coast live oak plots, currently observed level of mortality associated with *Phytophthora* in case plots was similar to the amount of recent (estimated 10 year) mortality from other causes (Figure 4). In other words, *Phytophthora* has doubled the mortality in case plots, whereas mortality in control plots has been increased by about 50% to date. If we assume that all trees with visible *Phytophthora* infections die within the next decade and that trees in severe decline due to other cause also die within this period, current host tree density would be reduced by nearly 50% in case plots and 33% in control plots. Although the total reduction in tree density considering all species would be substantially less (about 11% for both case and control plots), this anticipated mortality could substantially alter stand composition in the affected forests. Among all coast live oak plots, 39% of all trees were known *Phytophthora* hosts, but the percentage of host trees in these plots varied from 19% to 69% (Table 6). Hence, potential impacts of disease due to *Phytophthora* may vary widely within a given geographic area.

The effect on the tanoak stands we sampled is even more pronounced, because this species is both more susceptible to *Phytophthora* and exhibits relatively low rates of mortality due to other causes (Figure 4). All of the observed mortality in tanoak case plots and 69% of the mortality in tanoak control plots was related to *Phytophthora.* The mortality rates we observed, 22% in case plots and 20% in control plots, are well above the rates reported by Hunter (1997). In a stand in Mendocino County, he recorded 6% mortality among tanoaks <20 cm DBH and 9% mortality among tanoaks >20 cm DBH over a 14 year period (1981-1995). If all symptomatic trees in tanoak case plots die, the current host density in these stands would be reduced by 50%, the same amount calculated for coast live oak case plots. However, symptomatic host trees represent only 17% of the total tree density in the tanoak plots, and most of these are partially to completely

overtopped by a coast redwood or mixed conifer overstory. In these two study areas, even complete elimination of tanoak due to disease would have relatively minor effects on overall forest canopy cover, although stand composition and habitat characteristics would be greatly altered.

Previously reported water potential values for coast live oak and tanoak

Prior to initiating our study, we reviewed the existing literature on water relations of coast live oak and tanoak. Although various researchers have measured Ψ in both species (Table 14), only the methods used by Geary (1999) are comparable with the methods we used. Predawn water potentials have been measured in many other studies. These readings represent the highest water potentials a plant experiences during a 24 hr period and measure the degree to which water potentials can recover overnight. Although predawn Ψ typically is lower in water-stressed trees than in nonstressed trees, it does not directly indicate the maximum degree of water stress that develops during midday.

Midday leaf water potentials reported by some authors do not directly correspond to midday stem Ψ. Midday leaf water potentials measurement can be subject to large errors associated with post-excision leaf drying and within-leaf Ψ gradients unless transpiration is stopped by placing leaves in plastic bags prior to excision (McCutchan and Shackel 1992). Neither Griffin (1973) nor Knops and Koenig (1994) mention in their methods the use of plastic bags, so it is likely that their midday leaf Ψ measurement were influenced by these sources of error. Furthermore, since midday leaf Ψ readings would also be influenced by VPD and canopy shading, the lack of information on these parameters further constrains any data comparisons. In our study, variation in VPD accounted for nearly 1 MPa difference in stem water potential readings in unshaded trees (Figure 2).

Our average stem Ψ readings for tanoak at locations 9 and 12 (-0.66 and -1.0 MPa, respectively; Figure 1) are higher than October 1994 readings from Mendocino County and July 1994 readings from Napa County reported by Geary (1999) (Table 14). While this suggests that the trees we observed had lower levels of water stress than those observed by Geary (1999), we cannot determine how much of this difference could be due to differences in VPD, levels of canopy shading, or other factors that may influence stem Ψ. Future studies involving stem Ψ measurements in oak and tanoak need to account for these factors to permit meaningful comparisons between different studies.

Griffin (1973) showed that stand density had little effect on predawn leaf Ψ of coast live oaks growing on alluvial terraces where trees presumably had access to adequate reserves of soil moisture. Although most of the coast live oaks in our study were located on uplands and slopes, stem Ψ was not correlated with either total tree density or host tree density within plots. This could be due to relatively high levels of soil moisture levels in these areas. Alternatively, it is possible that stand densities in these areas have adjusted over time through natural mortality to the point that stand water use does not exceed available soil moisture supplies, at least in a year of near normal rainfall.

Previous studies conducted across more than one year have shown that plant Ψ in both tanoak and coast live oak can be influenced by rainfall amounts during the wet season. Griffin (1973) showed that predawn water potentials in coast live oak were considerably lower following a very dry year than following a very wet year (Table 14). He also noted that coast live oak is not drought deciduous and trees responded to the very dry 1967-68 rainfall season by failing to produce new foliage during the growing season. Geary (1999) reported that seedling and sapling

tanoaks in Mendocino county showed lower leaf water potentials and low-light stomatal conductances during a dry year (1994) than in a normal rainfall year (1993). Although the stem Ψ readings that we made in this study are probably at or near the maximum levels of water stress that the study trees experienced in 2000 (a near normal rainfall year), they do not necessarily represent plant water stress levels experienced in other years, and probably do not represent Ψ levels that existed at the time that infection occurred. Repeated observations of stem Ψ on trees taken in different seasons and years and more information on the epidemiology of *Phytophthora* sp. nov. will be needed to better understand the relationship between plant water status and disease risk.

Table 14. Xylem water potentials of coast live oak and tanoak reported in the literature. Values from this report are included in the table for comparison.

Species	Water potential (MPa)	Location	Notes	Citation
Q. agrifolia	Midday stem: -0.25 to -3.1 mean -1.4±0.61	Marin (9 locations) and Napa (1 location) Counties	31 Aug - 25 Sep 2000; 128 trees	this report
Q. agrifolia	Predawn: -1.22±.58 Midday:-2.09±0.49	Hastings Reserve, Monterey Co.	20 Sep - 5 Oct 1991; 63 trees	Knops and Koenig 1994
Q. agrifolia	Predawn:-0.41 to -3.14	Hastings Reserve, Monterey Co.	Mid Aug 1968, very dry year; lowest readings were from upland trees	Griffin 1973
Q. agrifolia	Predawn: -0.3 to -0.51	Hastings Reserve, Monterey Co.	Mid Aug 1969, very wet year	Griffin 1973
Q. agrifolia	Midday:-1.67 to -3.00	Hastings Reserve, Monterey Co.	between Jun 1968 and Sep 1970; 4 trees	Griffin 1973
Q. agrifolia	Predawn: -2.22 to -3.68	Jasper Ridge San Mateo County	Sep and Oct readings from interval 7 Sep 89 - 7 Oct 90; 8 scrubby *Q. agrifolia* on serpentine soil	Goulden 1996
Q. agrifolia	Predawn: -0.74 to -1.21	Jasper Ridge San Mateo County	Nov 1989 - Jun 1990	Goulden 1996
L. densiflorus	Midday stem: -0.425 to -1.65 mean -0.86±0.32	Muir Woods, Marin Co., Jack London SP, Sonoma Co.	20 and 28 Sep 2000; 22 trees	this report
L. densiflorus	Midday stem: mean -0.75 (July) to -1.4 (Oct)	Angelo Coast Range Reserve, Mendocino Co.	Jul to Oct 1994; 3-7 plants at each of 4 sites	Geary 1999
L. densiflorus	Midday stem: mean -2.3	Angwin, Napa Co.	Jul 1994; 5 plants	Geary 1999
L. densiflorus	Predawn: -0.3 to -0.5	Southwestern Oregon	monthly readings over 12-18 month period 1986 - 1988; 6 sprout clump saplings; little variation observed across seasons	Harrington, Pabst, Tappeiner 1994
L. densiflorus	Afternoon: -0.6 to -1.4 (stomata nearly closed at Ψ of -2.3)	Southwestern Oregon	lowest readings in summer	Harrington, Pabst, Tappeiner 1994

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DATA SHEET

Date: Location:

Subject tree number:				*CASE / CONTROL*	
species: Qa Ld		**# stems:**		**origin:** seed / sprout	
unshaded canopy percentage:		Plot canopy cover		open / dom / codom / int / overtop	
DBH stems>3cm			Phycanker count		
canopy dieback			Phyt % girdling		
decay impact					
thinning					
epicormics				**time:**	
midday stem ψ					
Plot slope	%		plot aspect	°	
Basal area (# trees "in")				BAF= m^2/ha	
	Qa	Qk	Ld	Nonhosts	
Density count				Qc R	Pm R
Phytop: no				Qd R	Ss R
early disease				Ql R	Man R
late disease				Uc R	
dead				Am R	
Others: decline				Ha R	
dead				Ac R	
pathogens/agent symptoms	Phytop. / H.thouar. / Inonotus / Phellinus / Ganoderma Laetiporus / Armillaria / white rot / canker rot / twig-stem canker / beetles				
Regen - density				woody understory cvr (0-6)	
Regen - % disease					
disturbance					