

# A Multiple Logistic Regression Model for Predicting the Development of *Phytophthora ramorum* symptoms in Tanoak (*Lithocarpus densiflorus*)<sup>1</sup>

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## Abstract

*Phytophthora ramorum* attacks tanoak (*Lithocarpus densiflorus*) in California and Oregon. We present a stand-level study examining the presence of disease symptoms in individual stems. Working with data from four plots in redwood (*Sequoia sempervirens*)/tanoak forests in Marin County, and three plots in Mendocino County, California, we evaluated a number of stem variables such as dbh, height, crown class, foliar condition and the presence of insect, fungal infection or disease symptoms for significance in multiple logistic regression analysis. We built a maximal model of variables and simplified this down following Akaike's Information Criterion. Our final model includes only variables significant at  $p = 0.01$ . We compare disease presence and mortality results in Marin stands with results collected in Mendocino County.

*Key words:* mortality, multiple logistic regression, *Phytophthora ramorum*, tanoak

## Introduction

*Phytophthora ramorum* has emerged as a major source of tanoak (*Lithocarpus densiflorus*) mortality in the redwood/tanoak forests of central California. In this forest system other species hosts of *P. ramorum* include madrone (*Arbutus menziesii*), California bay laurel (*Umbellularia californica*), bigleaf maple (*Acer macrophyllum*), huckleberry (*Vaccinium ovatum*) and redwood (*Sequoia sempervirens*) (Rizzo and others 2002a). While the pathogen seems to cause rapid mortality in madrone and tanoak, infection appears to be limited to leaves in these other hosts (Rizzo and Garbelotto 2003).

Unlike coast live oak which is limited to stem infections, tanoak are also susceptible to foliar *P. ramorum* infection (Rizzo and Garbelotto 2003, Rizzo and others 2002b). The ability of tanoak to potentially support sporulation might diminish the role of other foliar host species as vectors in redwood/tanoak stands. To date, published reports on the dynamics of the disease in redwood/tanoak stands are limited.

This study builds a multiple logistic regression model using individual stem

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<sup>1</sup> This paper was presented at the Redwood Science Symposium: What does the future hold? March 15-17, 2004, Rohnert Park, California.

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attributes. Variables initially considered for inclusion in the model are listed in *table 1*. We developed our binary response variable using the results from our last stand survey in June 2003 including only trees that showed no *P. ramorum* symptoms in our July 2001 survey. The model that we have built enables us to assess the influence of individual tree attributes on *P. ramorum* infection probabilities for individual tanoak stems. We utilize the model to develop suggestions for management action.

**Table 1**—List of stem attributes collected and included in the maximal model.

Species
height, diameter, crown class
percent live foliage, foliar condition
presence/absence of bark and ambrosia beetles
stem growing individually or attached below breast height
presence of infection on neighboring stem attached below breast height

## Methods

The study area is located on Marin Municipal Water District lands on the East Slope of Bolinas Ridge above Kent Lake reservoir (38.0° N, 122.7° W). The study plots are at elevations between 110 and 270 m. Soils of the area include Dipsea and Barnabe very gravelly loams (Kashiwagi 1985). The forest canopy is stratified with redwood and Douglas-fir (*Pseudotsuga menziesii*) in the upper stratum and tanoak, madrone, and California bay occupying the layer beneath. Annual precipitation averages between 750 and 1250 mm. Average temperature is 12 °C (Kashiwagi 1985).

In the summer of 2001 we established the four Marin plots containing an average of 410 stems greater than 4cm dbh. These plots range in size from 50 m by 60 m (0.32 ha) to 50 m by 80 m (0.4 ha). The density of tanoak stems in these stands ranged between 840 and 1020 stems/ha. We established the three Jackson plots 0.26, 0.29 and 0.30 ha, respectively, in February of 2002. Tanoak stem densities in these plots ranged between 890 and 1090 stems/ha. Within each plot we used a Laser-Ace 300 and Haglof distance measuring device to map all stems greater than 4 cm dbh with a relative precision of ±10 cm. In addition to recording species, diameter, height, and crown class we visually estimated foliar condition and documented the presence/absence and age of bark and ambrosia beetle infestation, presence/absence of fungi infestation including *Hypoxylon thouarsianum*, presence/absence and location of bleeding areas, presence/absence of stem cankers beneath bark wounds and the presence/absence of apparent foliar and twig infections consistent with *P. ramorum* infection. These plots were revisited and each tree assessed four times over the three year period of the study.

To better understand the dynamics of tanoak mortality in the absence of *P. ramorum* we established and mapped three plots in *P. ramorum* free stands at Jackson Demonstration State Forest. The three plots are located in the Little North Fork of the Big River watershed (39.35° N, 123.65° W at 180 m) and (39.35° N, 123.71° W at 240 m) and the Upper Parlin Creek (39.40°N, 123.66°W at 240 m) in Mendocino County. The soils of the basins are well-drained clay-loams, one to two m in depth and derived from Franciscan greywacke sandstone and weathered, coarse-grained shale of the Cretaceous Age. Ninety percent of the annual precipitation of 1200 mm falls from October through April (Rittiman and Thorson 1987).

To test the accuracy of our field diagnosis and assess our ability to differentiate between bleeding areas related to *P. ramorum* cankers and those due to other causes we randomly selected 36 stems from our population of stems with bleeding areas. We submitted these samples for PCR and culture analysis. Of the 36 samples, our field assessment classified 19 as *P. ramorum* infected and 17 as uninfected with bleeding areas that were due to causes unrelated to *P. ramorum*. The results of the PCR analyses confirmed our field diagnoses with 100 percent consistency.

We developed a multiple logistic regression model to assess the relationship between stem attributes and the probability of individual stems developing *P. ramorum* symptoms. We coded these stems with “0” or “1” depending on whether they developed a *P. ramorum* related bleeding stem canker or foliar symptoms between the initial, 2001, and final, 2003, stand surveys. For each stem the percent live foliage, foliar condition and presence/absence of bark variables date from the prior year's survey. Thus if a tree became symptomatic in 2002 its relevant vegetation and invertebrate data from the 2001 survey were entered in the model. For trees that remained symptom free throughout the course of the study, data from the survey immediately prior to the final June 2003 survey were entered in the model. We arcsine transformed foliar data. Prior to including predictor variables in the maximal model we examined them for correlation and used stepwise deletions following the Akaike Information Criterion to arrive at an initial minimal adequate model (Akaike 1973). We then tested for non-linear and interaction effects and compared the resulting models with the initial minimum adequate model to determine whether these non-linear or interaction explanatory variables led to a significant increase in the explanatory power of the model. We performed all statistical analyses using SPLUS 6.2 statistical software.

## Results

In the three Mendocino plots the average density of tanoak stems was 1040 stems/ha. In our initial survey in February 2002 we found an average of 131 dead stems/ha (11 percent of stems). No additional stem mortality occurred in those plots between the initial survey in January of 2002 and final survey in August of 2002. In that time period no stems exhibited symptoms of *P. ramorum* infection. By comparison in the Marin plots mortality climbed from an average 151 to 283 tanoak stems/ha between July 2001 and June 2003, an average increase of 15 to 29 percent of tanoak stems over the course of the study. Symptom incidence rose from an average 112 to 183 symptomatic tanoak stems/ha, an average increase of 12 to 20 percent of tanoak stems. In *table 2* we provide a summary of stem counts, disease incidence and stem mortality by plot.

We list our multiple logistic regression model parameter estimates for the binary disease outcome in *table 3*. The presence or sign of bark beetle activity on the stem in the previous year contributes more to the probability of becoming symptomatic than the other variables in this model. Of the 24 trees that became symptomatic between July of 2002 and June of 2003, four had insect activity in 2002. Of the 29 trees that became symptomatic between February of 2001 and July of 2002, seven had signs of insect activity in 2001. Signs of insect activity in tanoaks are rare (McPherson and others 2002). Of the 888 stems included in the model only 112 showed signs of insect activity throughout the study. The percent live foliage of the previous year was also highly significant.

**Table 2**—A summary of plot sizes, stem counts, disease incidence, and stem mortality by plot and sampling date.

Plot			Sampling date					
			July 2001	Jan 2002	Feb 2002	July 2002	Aug 2002	June 2003
<b>Marin county</b>								
<b>11</b>	0.42 ha.	dead tanoak stems	30		61	65		78
		425 stems	percent tanoak stems that are dead	9%		18%	19%	
	348 tanoak stems	density of dead tanoak per ha.	72		147	157		188
		839 tanoak stems/ha.	symptomatic tanoak stems	48		66	80	
		percent tanoak stems that are symptomatic	14%		19%	23%		26%
		density of symptomatic tanoak stems per ha.	116		159	193		217
<b>12</b>	0.36 ha.	dead tanoak stems	34		77	86		100
		397 stems	percent tanoak stems that are dead	10%		23%	25%	
	338 tanoak stems	density of dead tanoak per ha.	95		215	241		280
		945 tanoak stems/ha.	symptomatic tanoak stems	33		47	52	
		percent tanoak stems that are symptomatic	10%		14%	15%		17%
		density of symptomatic tanoak stems per ha.	92		131	145		159
<b>13</b>	0.33 ha.	dead tanoak stems	56		89	91		107
		379 stems	percent tanoak stems that are dead	17%		28%	28%	
	321 tanoak stems	density of dead tanoak per ha.	172		274	280		329
		988 tanoak stems/ha.	symptomatic tanoak stems	40		58	65	
		percent tanoak stems that are symptomatic	12%		18%	20%		21%
		density of symptomatic tanoak stems per ha.	123		178	200		209
<b>14</b>	0.36 ha.	dead tanoak stems	95		108	115		121
		439 stems	percent tanoak stems that are dead	26%		30%	32%	
	365 tanoak stems	density of dead tanoak per ha.	266		302	322		338
		1021 tanoak stems/ha.	symptomatic tanoak stems	42		44	47	
		percent tanoak stems that are symptomatic	12%		12%	13%		15%
		density of symptomatic tanoak stems per ha.	117		123	131		148
<b>Mendocino county</b>								
<b>21</b>	0.26 ha.	dead tanoak stems		26			26	
		450 stems	percent tanoak stems that are dead		10%		10%	
	260 tanoak stems	density of dead tanoak stems per ha.		100			100	
		1000 tanoak stems/ha.	symptomatic tanoak stems		0		0	
<b>22</b>	0.29 ha.	dead tanoak stems		27			27	
		380 stems	percent tanoak stems that are dead		10%		10%	
	285 tanoak stems	density of dead tanoak stems per ha.		93			93	
		983 tanoak stems/ha.	symptomatic tanoak stems		0		0	
<b>23</b>	0.3 ha.	dead tanoak stems		58			58	
		506 stems	percent tanoak stems that are dead		18%		18%	
	328 tanoak stems	density of dead tanoak stems per ha.		193			193	
		1093 tanoak stems/ha.	symptomatic tanoak stems		0		0	

**Table 3**—Multiple logistic regression model for binary disease outcome—the probability of individual stems developing *P. ramorum* symptoms.

Variable	Value	Std. Error	F value	Prob (F)
Presence or sign of bark beetle activity on the stem in the previous year	0.87	0.22	16	<0.0001
Percent live foliage the previous year	-1.8	0.38	51.5	<0.0001

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Null deviance of the model: 641 on 887 degrees of freedom

Residual deviance: 574 on 880 degrees of freedom

## Discussion

The multiple logistic regression model enables us to evaluate the effect of stem attributes on the probability that an individual stem would develop stem or foliar symptoms through the period of the study. We examine the role of the two variables in the model and reflect on the variables that were not significant. We also discuss the potential role of the variables not measured in this study.

The two variables in this model may be indicators of undetected infection rather than predictors of symptom development. Both the “presence or sign of bark beetle activity in the previous sampling period” variable and the “percent live foliage the previous year” were highly significant. Unfortunately the design of this study does not enable us to determine the source of their significance.

As previously noted, beetle activity in tanoaks is rare. The association between beetle activity in the year preceding the appearance of symptoms indicates a potential role for beetles in the spread of the disease. It is also possible that *P. ramorum* infected tanoaks may become susceptible to beetle infestation prior to exhibiting visible stem canker bleeding. McPherson and others (2002) note that bark beetles are rare on living Coast live oak (*Quercus agrifolia*) in the absence of *P. ramorum* related bleeding stem cankers. Their study suggests a weak association between bleeding stem cankers in tanoak and the subsequent appearance of beetle infestation.

While our study does not provide proof of this assertion we suggest that “decrease in percent live foliage in the preceding year” variable in the model is more likely to be an artifact of undetected infection rather than a predictor of either visible foliage or bleeding stem canker symptom development. Flagging branch tips and scattered leaf death often precede the appearance of bleeding cankers (McPherson and others 2002). Thus the significance of decreased percent foliage observed in the year previous to the actual detection of *P. ramorum* symptoms has two likely causes. First, while we were able to detect a decrease in foliar cover we might have missed signs of foliar infection at crown height. We may have also missed signs of infection on abscised leaves at the base of all stems despite our efforts. Second in the initial phases of infection *P. ramorum* related stem cankers may sufficiently obstruct xylem flow to reduce foliar cover prior to causing the development of bleeding characteristics.

The dbh of a tree was not a significant predictor for the development of symptoms under our model nor were the height of a stem or its location in the

canopy. Stems that grew individually or clumped were not significantly different in their probability of infection. Uninfected stems growing attached below breast height to *P. ramorum* infected stems were also no more likely to become infected. While we did no testing for the genetic relationships between our stems these results indicate that such testing may not be necessary.

It is clear that multiple factors contribute to the probability of tanoak stems becoming symptomatic. Our study compares mortality rates between Marin and Mendocino County. Mortality remained unchanged for the first two years of the study in the un-symptomatic Mendocino stands. By comparison mortality increased in all four Marin County plots. We find it important to acknowledge that our Marin plots represent a small portion of the possible area for testing. We acknowledge that we tested a limited number of tree characteristics (dbh, crown class) and did not measure a number of potentially important variables including seasonal water stress, stem water potential, age, root mass, leaf area, and genetics. The most important contribution of our model is the indication it provides of the stem factors that do not affect the probability of a stem becoming symptomatic. The significance of beetle presence in the sampling period prior to detecting symptoms suggests that the matter should be researched more thoroughly.

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