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Forest Ecology and Management 213 (2005) 71–89
Forest Ecology and Management
www.elsevier.com/locate/foreco

Sudden oak death in California: Disease progression in oaks and tanoaks

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Received 6 December 2004; received in revised form 23 March 2005; accepted 25 March 2005

Abstract

Sudden oak death (SOD), caused by *Phytophthora ramorum*, is killing oaks and tanoaks in the Coast Ranges of California, from Monterey County to Humboldt County. In March 2000, 20 disease progression plots were established in Marin County, California, to characterize the progress of disease symptoms, and to determine the fidelity of the association of three or more bark and ambrosia beetle species (Coleoptera: Scolytidae) with diseased oaks and tanoaks. Symptoms of sudden oak death and signs of associated organisms were recorded from coast live oaks (*Quercus agrifolia*), California black oaks (*Q. kelloggii*), valley oaks (*Q. lobata*), and tanoaks (*Lithocarpus densiflorus*), four times per year, from March 2000 through March 2003. Symptoms and signs in *Q. agrifolia* progressed from bleeding, to infestation by scolytid beetles, to the development of fruiting structures of the fungus *Hypoxylon thouarsianum*. Mortality of symptomatic trees increased from 2000 to 2003 as follows: *Q. agrifolia* ($n = 668$), 5.8–17.4%; *Q. kelloggii* ($n = 53$), 3.8–9.4%; and *L. densiflorus* ($n = 164$), 8.3–22.2%. All 31 *Q. lobata* remained asymptomatic. From 2000 to 2003, bleeding trees were 25.0–23.6% of living *Q. agrifolia*, 15.5–25.0% of *Q. kelloggii*, and 39.0–62.4% of *L. densiflorus*. Scolytid beetles colonized more than 95% of the living symptomatic *Q. agrifolia* that subsequently died. Same-symptom cohorts were followed from March 2000 through March 2003. In the asymptomatic *Q. agrifolia* cohort, 12.0% developed bleeding by 2003. For the bleeding only cohort, 22.7% of *Q. agrifolia* died, but 73.5% of the beetle-colonized bleeding cohort died. Bleeding developed in 40.9% of the initially asymptomatic *L. densiflorus* cohort. By 2003, 24.6% of the initially bleeding *L. densiflorus* cohort had died. Both Weibull and Cox Proportional Hazards regression were

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0378-1127/$ – see front matter
doi:10.1016/j.foreco.2005.03.048

used to model cohort survival. The median survival time estimated by Weibull regression models declined rapidly by disease category (asymptomatic, bleeding only, bleeding with beetles), from 29 to 2.7 years for *Q. agrifolia*, and from 12.6 to 2.9 years for *L. densiflorus*. By 2003, structural bole failure had occurred in 21.5% of the *Q. agrifolia* that were bleeding in 2000, 93% of which had ambrosia beetle tunnels at the breakage point. For both *Q. agrifolia* and *L. densiflorus*, health failure analysis modeled by Weibull regression found a greater probability of developing sudden oak death for trees with larger stem diameters. Beetles were also positively correlated with larger diameter bleeding *Q. agrifolia*.

Keywords: Coast live oak; Tanoak; California black oak; Sudden oak death; *Phytophthora ramorum*; Scolytidae; Survival analysis

1. Introduction

Phytophthora ramorum, the pathogen that causes sudden oak death (SOD), infects a number of woody plant species in mesic forested habitats in coastal California and southwestern Oregon (Garbelotto et al., 2003; McPherson et al., 2000). The forests where this disease is presently found are classified as coastal oak woodland, montane hardwood, montane hardwood-conifer, redwood, and Douglas-fir types (Mayer and Laudenslayer, 1988). The death of the foliage of mature tanoaks (*Lithocarpus densiflorus*) was first observed in 1994 at the urban-wildland interface in Marin County, California (Svihra, 1999a), and subsequently in coast live oaks (*Quercus agrifolia*) in 1995 (Svihra, 2001). By the late 1990s, extensive mortality in these widely distributed species was reported in disjunct forests and woodlands throughout the Coast Ranges of California, from Big Sur in Monterey County, through the Santa Cruz Mountains, and north into Marin and Sonoma Counties. In 2000, a previously undescribed *Phytophthora* that had been isolated from bleeding bark cankers on *Q. agrifolia* and *L. densiflorus* in Marin County, California, was shown to produce the symptoms and signs of sudden oak death in both species (Rizzo et al., 2002). The same pathogen, *P. ramorum*, was previously isolated from ornamental *Rhododendron* species in The Netherlands and Germany (Werres and Marwitz, 1997; Werres et al., 2001), and is now recognized as the cause of sudden oak death (Rizzo et al., 2002).

The primary symptom of sudden oak death is the production of a viscous red to black exudate from bark cankers, typically on the lower trunk, referred to as bleeding. The bleeding areas on such trees are often colonized by bark and ambrosia beetles (Svihra, 1999a), and may exhibit the fruiting bodies (sporophores) of the native endophytic fungus, *Hypoxylon thouarsianum* (McPherson et al., 2000; Svihra, 2000). Bark and ambrosia beetles (Coleoptera: Scolytidae) are known to colonize stressed trees in a number of coniferous forests (Furniss and Carolin, 1977; Waters et al., 1985), but selective attacks on the bark overlying cankers has not been reported previously for any of the insects observed to colonize oaks that have root diseases or stem cankers (Chamberlain, 1958; Furniss and Carolin, 1977).

In natural environments in coastal California, *P. ramorum* is known to parasitize species in 12 plant families, including Aceraceae, Anacardiaceae, Betulaceae, Caprifoliaceae, Cupressaceae, Ericaceae, Fagaceae, Hippocastanaceae, Lauraceae, Pinaceae, Rhamnaceae, and Rosaceae (Garbelotto et al., 2003). The degree of pathogenicity varies by host species; in most, the disease causes foliar blights and twig cankers, with some hosts apparently serving as disease reservoirs (Rizzo and Garbelotto, 2003). The only large-scale mortality known to be due to stem cankers is in the red oaks and in *L. densiflorus*.

Three of the susceptible oaks, *Q. agrifolia*, California black oak (*Q. kelloggii*), and Shreve oak, (*Q. parvula* var. *shrevei*), are in the Erythrobalanus (red oak) subgenus. Canyon live oak (*Q. chrysolepis*), which is in the Protobalanus subgenus, is the other known host oak species native to California (Murphy and Rizzo, 2003). Although the susceptibility of most other oaks is not known, no mature North American species in the Lepidobalanus (white oak) group, which includes valley oak, (*Q. lobata*), have been reported to be hosts. The relationship between *Quercus* and the primarily southern Asian genus *Lithocarpus*, of which *L. densiflorus* is the sole New World representative, is complex and poorly defined.

A large number of the woody plants in these forests are *P. ramorum* hosts, but in most, the resulting diseases are not fatal. Oaks and tanoaks have ecological impacts out of proportion to their representation in these habitats. In addition to their importance as the principal (or only) nut-producing species, a number of birds, mammals, insects, and fungi are dependent on these trees. Understanding of the etiology of this disease, as modified through interactions with secondary organisms, should lead to improved predictions of its consequences to these ecosystems and to better management decisions.

Monitoring plots were established in early 2000 to characterize the sequence of symptom progression, with the goal of modeling rates of change in diseased trees. No causal agents had been identified, the association with beetles lacked a quantitative basis, and no trees had been monitored long enough to confirm temporal relationships among signs and symptoms. The three primary objectives of the study were to: (a) determine the strength of the association of the characteristic bleeding on the main stem in *Q. agrifolia*, *Q. kelloggii*, and *L. densiflorus* and infestation by ambrosia and bark beetles and the production of *H. thouarsianum* sporophores; (b) determine whether attacks by scolytid beetles on symptomatic trees resulted in acceleration of tree death and subsequent stem failure; and (c) estimate the impact of sudden oak death in the forests where the plots were located.

2. Materials and methods

2.1. Study sites

Disease progression plots were located to encompass a variety of forest types. Several factors were considered in plot selection. These included the dominance of the principal host tree species of interest *Q. agrifolia* (eight plots), *L. densiflorus* (two plots), history of past fires (three plots), stand structure, and the diversity of host and non-host tree species within these sites. In the absence of standing dead or obviously failing trees, the presence of the disease in a plot could only be detected by inspection. Thus, all plots were placed in watersheds where the disease had been reported, but without prior knowledge of its presence within each site. Plot size and shape were delimited by physical boundaries; including trails, topography (ravines and ridge tops), and such natural vegetation cover transitions as open fields and increased density of shrubs. This selection process led to plots that varied considerably in the numbers of stems and proportions of host trees that were symptomatic. Ten plots were established at each of two protected forests, one in China Camp State Park and one in land managed by Marin Municipal Water District, near Mt. Tamalpais State Park. The number of host trees per plot ranged from 35 to 84. Plots were chosen to contain primarily *Q. agrifolia* and *L. densiflorus*, with *Q. kelloggii* and *Q. lobata* occurring as relatively minor components.

China Camp State Park is located on the southwestern shore of San Pablo Bay, (latitude = 38.00059, longitude = −122.48514) near the city of San Rafael. Elevation varies from near sea level to 290 m. The dominant overstory forest trees are *Q. agrifolia*, *Q. kelloggii*, bay laurel (*Umbellularia californica*), and madrone (*Arbutus menziesii*). Other overstory trees that constitute significant portions of the forest flora and that are locally abundant include *Q. lobata*, blue oak (*Q. douglasii*), California buckeye (*Aesculus californica*), Douglas-fir (*Pseudotsuga menziesii*), big leaf maple (*Acer macrophyllum*), *Eucalyptus* spp., and coast redwood (*Sequoia sempervirens*). The vegetation cover varies considerably, comprising open fields, dense shrub thickets, and relatively open, oak-dominated closed canopy forests. The coastal oak woodland, montane hardwood, and montane hardwood-conifer forest types (Mayer and Laudenslayer, 1988) are represented in China Camp plots. Common shrubs, small trees, and vines include manzanita (*Arctostaphylos manzanita*), toyon (*Heteromeles arbutifolia*), hazel (*Corylus cornuta*), buckeye (*Aesculus californica*), coffeeberry (*Rhamnus californica*), huckleberry (*Vaccinium ovatum*), rhododendron (*Rhododendron macrophyllum*), poison oak (*Toxicodendron diversilobum*), and honeysuckle (*Lonicera hispidula*). With the exception of the white oaks, *Q. douglasii* and *Q. lobata*, and *Eucalyptus* spp., these woody plants have all been confirmed as hosts of *P. ramorum* (Davidson et al., 2003; Garbelotto et al., 2003).

The Marin Municipal Water District (MMWD) watershed (latitude = 37.5721, longitude = −122.3636), NE of Mt. Tamalpais State Park, is generally at a

higher elevation than China Camp State Park. The elevation of the research plots varies between 190 and 270 m. In addition to the tree and shrub species found in China Camp State Park, *L. densiflorus* and dense stands of *S. sempervirens* are present in MMWD. The forest types represented include those in China Camp, as well as the redwood and Douglas-fir forest types (Mayer and Laudenslayer, 1988).

Both sites have a temperate marine climate, with potential for fog at least part of the day for much of the year. Annual rainfall in China Camp State Park averaged 91 cm between 1948 and 2000. Rainfall in MMWD averaged 132 cm per year between 1979 and 1996. The annual mean temperature range in the city closest to both locations, San Rafael, is 8.9–21.4 °C.

Three plots are greater than 50-m from hiking or bicycling trails. One plot is adjacent to a campsite, in 15 plots a trail defines one edge, and two plots are within 20-m of a trail. In summer 2001, one plot in MMWD was burned. All the trees in the burned plot and an additional 18 symptomatic *Q. agrifolia* that were removed in 2001 from a plot in China Camp State Park as hazards were deleted from further analysis.

2.2. Plot characteristics

Each oak and *L. densiflorus* stem greater than 5 cm diameter at breast height (dbh) was labeled with an aluminum tag. The coordinates of most trees were recorded using a Trimble GeoXT DGPS (Differential Global Positioning System) unit. Each stem that was separated from other stems at 1 m above the soil line was considered to be an individual tree. Although this treatment effectively considers genetically identical stems as separate individuals, it is consistent with the observation that bleeding often appears to be distributed independently on the stems of multiple stem trees. Plot size varied as a function of natural landscape features and ranged from 320 to 3600 m^2, with a mean area of 1234 m^2 (S.E. = 199 m^2). Survival of *Q. agrifolia* seedlings and saplings in the study area was poor in the understory below mature trees, whereas *L. densiflorus* were abundant in the understory. *Q. agrifolia*-dominated plots had few individuals smaller than about 10 cm dbh, but the size distribution of the *L. densiflorus* included many smaller trees.

Trees were monitored at approximately 3-month intervals, beginning in March 2000. The resampling frequency allowed the verification of symptoms for individual trees in which characterization of symptom states was uncertain on earlier dates.

2.3. Symptom evaluation

Trees were evaluated on the basis of the observed symptoms and signs that are associated with sudden oak death in oaks and *L. densiflorus* (Davidson et al., 2003; McPherson et al., 2000; Rizzo et al., 2002). The most consistent symptom of *P. ramorum* infection in these species is bleeding. By this definition, an asymptomatic tree, while not bleeding, could show other symptoms of decline. A recently isolated pathogen, *P. nemorosa*, causes bleeding cankers in oaks and in *L. densiflorus*, although it appears to be much less consistently fatal in oaks (Hansen et al., 2003). Symptom-based disease estimates may therefore overestimate the true incidence of *P. ramorum* in these plots. Each tree was visually rated for presence or absence of bleeding on the main stem. Bleeding was rarely observed on lateral branches. Assessment of bleeding was usually straightforward when the presence of the exudate was obvious. Although heavy rain can remove the exudate, the bark below bleeding areas often remained stained for months. In order to standardize symptom evaluation among observers, only bleeding that was noted on consecutive monitoring dates was recorded. Mosses and lichens below bleeding zones may be stained dark brown by this exudate, signaling the presence of a bleeding canker. Oak trees also produce exudate on the bark in response to injuries and other pathogens, such as *Armillaria* spp. (Sinclair et al., 1987) and other *Phytophthora* spp. (Mercetich et al., 1977; Tainter et al., 2000). Bleeding can be confused with bacterial wetwood, found in oaks and many other hardwoods, characterized by malodorous and watery seeping associated with branch stubs, bark fissures, and old wounds (Hartley et al., 1961). However, wetwood seeping can be readily differentiated from the bleeding associated with *P. ramorum* infection, which dries to a hard consistency with a distinct oak odor. Beetle tunneling has not been observed to be associated with wetwood.

Current methods for confirming *P. ramorum* infection in suspect plants require the removal of tissue samples for culturing or DNA amplification

(Blomquist and Kubisiak, 2003). Since wounding the bark potentially facilitates fresh *P. ramorum* inoculation, possibly attracting saprotrophic insects and introducing pathogens, thus affecting the natural course of events being monitored, the decision was made to not cut into bark to sample tissue.

The evaluation of sudden oak death was based on symptoms alone. The tunneling of bark and ambrosia beetles was detected by the presence of white and reddish brown boring dust on the bark surface. Within the first year following the appearance of bleeding, beetle tunneling is restricted to the bark directly overlying bleeding cankers. The tunneling activity of these insects occurs primarily during two periods, from March through June and from August through October (P. Svihra, unpublished data). When boring dust is not fresh, its presence from previous flight seasons can often be observed matted in moss, bark crevices, and spider webs. Also, entrance and exit holes (approximately 1 mm diameter) produced by these beetles can be seen with careful observation.

The sporophores of *H. thouarsianum* were recorded as present when they occurred on the main stem of a living tree. The ecology of this endophyte is not well understood. The presence of reproductive structures is the only reliable way to confirm the presence of this otherwise cryptic fungus. In the absence of sudden oak death, sporophores are typically observed on standing dead and fallen trees, dead branches on living trees, and the dead portions of the stems of standing, live trees.

Every time trees were assessed, the presence or absence of bleeding, beetle frass, and fruiting structures of *H. thouarsianum* were recorded. A tree was considered dead once its foliage had turned brown. Green sprouting from the root crown was observed in many of these trees after all the existing foliage was brown, but the tree was never observed to recover.

2.4. Structural failure

The structural failure of *Q. agrifolia* on the main bole was evaluated for each of the 13 monitoring dates, from March 2000 through March 2003. The presence of scolytid beetle tunneling, the presence of *H. thouarsianum* sporophores, and whether leaves were green or brown at the time the breakage occurred were recorded. Only trees exhibiting structural failure on the main bole were counted as failures.

2.5. Data analysis

Although observations were recorded at 3-month intervals, the observed low rate of change in symptom progression led to the decision to analyze the data in 1-year increments (March 2000, March 2001, March 2002, and March 2003) to determine the incidence of: (a) living trees showing bleeding cankers only; (b) the presence of bleeding cankers plus beetle activity; (c) the presence of bleeding cankers, beetles, and *H. thouarsianum* sporophores together; and (d) the presence of beetles and *H. thouarsianum* sporophores separately, in the absence of bleeding cankers. Dead trees that exhibited evidence of bleeding before death, and those trees that died without the bleeding symptom were analyzed separately.

2.5.1. Cohorts

Cohort analysis (Law, 1981) was applied to same-symptom cohorts of *Q. agrifolia* and *L. densiflorus* that were defined at the start of the study in March 2000 to estimate the duration of each disease stage, the proportion of the population affected, and survival through March 2003 within each cohort. Similar approaches have been used to follow the progress of air pollution injury in Southern California forests (Cobb and Stark, 1970) and pitch canker in urban forests of central California (Storer et al., 2002). For *Q. agrifolia*, cohort categories and numerical codes used for the survival analyses were: asymptomatic (1); bleeding only (2); bleeding with beetle colonization (3); bleeding with beetle colonization and *H. thouarsianum* fruiting bodies (4); and dead (5). The *L. densiflorus* cohort categories were asymptomatic (1); bleeding only (2); bleeding with beetle colonization, with or without *H. thouarsianum* fruiting bodies (3); and dead (4). The numbers of bleeding trees colonized by beetles and those that also showed *H. thouarsianum* sporophores were not analyzed separately, because few bleeding *L. densiflorus* in the plots exhibited these secondary organisms. There were insufficient numbers of *Q. kelloggii* for a similar analysis. The symptom states employed here could only be precisely defined for the asymptomatic cohorts, since the infection date, the date of first bleeding, and the date of beetle colonization were all unknown. However, the timing of changes in status was known within three months for all trees.

These analyses yielded the numbers of trees that had left the initial category and entered another, at 1-year intervals. Thus, for the initially asymptomatic *Q. agrifolia* cohort, the categories for subsequent years included: asymptomatic trees; trees that developed bleeding only; trees that developed bleeding and were colonized by beetles; trees with bleeding, beetles, and *H. thouarsianum* sporophores; and dead trees (with and without bleeding). For the bleeding only cohort, subsequent categories were: bleeding with beetles, bleeding with beetles and *H. thouarsianum* sporophores, and dead trees. Trees that were initially categorized as bleeding with beetles could progress to development of *H. thouarsianum* sporophores and to death. Trees that were categorized as bleeding with beetles plus *H. thouarsianum* sporophores could only progress to death.

The Weibull survival regression model (parametric model) and the Cox proportional hazard (PH) model (semi-parametric model) (Lee and Wang, 2003) were both used to analyze survival time for each of the *Q. agrifolia* and *L. densiflorus* cohorts described above. SAS 8.2 and SPSS release 10.0 were used to estimate the parameters and prepare graphic displays. The variables that were considered as explanatory variables to be included in the regression models were tree category (defined above), dbh in year 2000, and for the oaks only, site (China Camp and MMWD). Akaike's information criterion (AIC) was used to decide whether a variable should be included in the analysis (Akaike, 1974).

The Cox PH model is the most commonly used model for survival analysis. This is because it does not require knowledge of the underlying distribution of the survival scores. However, this model cannot predict survival beyond the time frame of the input data. Because this study has only three years of data, an insufficient time interval to envision possible survival times of 10–30 years, a model using the Weibull probabilistic distribution was also developed. This two-parameter distribution is most commonly used because of the different distributional shapes that it can take just by changing the values of these two parameters. Thus, the results of the survival analysis should be viewed in a critical manner in lieu of additional years of data.

The observation period in this study was relatively short and the models differ in both scope and limitations. The survival functions for both models are displayed to help readers reach their own conclusions about the reliability of the projected results.

2.5.2. Relationship between stem diameter and bleeding

The relationship between stem diameter and bleeding status was analyzed using *t*-tests (SAS, 2002) to compare the diameters (dbh) of living symptomatic and asymptomatic trees in 2000. For *Q. agrifolia* and *L. densiflorus*, the analysis was performed for the populations of living trees at the March 2000 starting date, and then for March of each subsequent year, while for *Q. kelloggii*, these trees were evaluated for only 2000 and 2003. The relationship between stem diameter and bleeding status for dead trees, including those that were dead at the start of the study, was evaluated using *t*-tests. Symptomatic and asymptomatic dead trees were analyzed separately. Stem diameter was also analyzed as a predictor of beetle colonization of bleeding *Q. agrifolia* for 2000 only. Weibull regression was used in a health analysis to define the relationship between tree diameter and the development of bleeding for *Q. agrifolia* and *L. densiflorus*.

2.5.3. Association of beetles with bleeding trees

The association of scolytid beetle activity with bleeding was analyzed for *Q. agrifolia*, *Q. kelloggii*, and *L. densiflorus* using log-likelihood ratio tests (SAS, 2002). Analyses were run on *Q. agrifolia* and *L. densiflorus* at each of the four annual sampling dates. *Q. kelloggii* were analyzed only for 2000 and 2003.

3. Results

3.1. Plot characteristics

The mean diameter at breast height of *Q. agrifolia* ranged from 21.5 to 44.5 cm on a plot (Table 1). On a plot basis, the incidence of both living and dead trees that had symptoms consistent with sudden oak death ranged from 0 to 65% in 2000 (Fig. 1). By 2003, this percentage had increased in all plots that had bleeding trees in 2000. For *L. densiflorus*, the mean diameter at breast height ranged from 12.2 to 20.2 cm per plot

Table 1
Abundance of oaks and tanoaks by plot and the mean diameter for species in plots with four or more stems each[a]

Plot	Coast live oak	Mean DBH	Black oak	Mean DBH	Valley oak	Mean DBH	Tanoak	Mean DBH
C-1	65	29.6 ± 1.6	4	50.8 ± 5.5	4	39.4 ± 4.3	0	0
C-2	48	37.4 ± 2.0	0		5	29.0 ± 3.5	0	0
C-3	69	29.6 ± 1.5	6	46.6 ± 3.3	9	25.4 ± 2.5	0	0
C-4	34	44.5 ± 4.8	4	40.0 ± 7.4	4	32.4 ± 3.8	0	0
C-5	40	39.1 ± 4.0	0		0		0	0
C-6	40	34.6 ± 2.0	5	47.8 ± 9.0	2		0	0
C-7	38	33.0 ± 2.5	0		5	31.5 ± 4.1	0	0
C-8	52	25.3 ± 1.2	0		0		0	0
C-9	45	31.4 ± 1.6	0		0		0	0
C-10	35	27.9 ± 2.2	0		0		0	0
M-1	0	0	0		0		46	18.6 ± 2.1
M-2	38	38.7 ± 3.5	12	38.1 ± 2.4	2		0	0
M-3	24	23.4 ± 2.2	13	27.0 ± 2.8	0		35	20.2 ± 2.1
M-4	11	37.4 ± 7.4	1		0		28	12.2 ± 0.9
M-5	40	27.9 ± 2.3	0		0		0	0
M-6	33	41.3 ± 2.1	5	38.6 ± 10.6	0		0	0
M-7	36	21.5 ± 2.0	0		0		14	19.6 ± 3.5
M-8	1	–	0		0		44	13.3 ± 1.7
M-9	45	32.3 ± 1.9	2		0		1	0
M-10	37	25.1 ± 1.5	1		0		14	12.3 ± 1.9

Means (cm) ± S.E.
[a] Plots C-1 to C-10 are in China Camp State Park. Plots M-1 to M-10 are in Marin Municipal Water District. The diameter values are for March 2000.

(Table 1). Both *Q. kelloggii* and *Q. lobata* were present as relatively minor components, averaging approximately five stems for each species in the plots where they occurred.

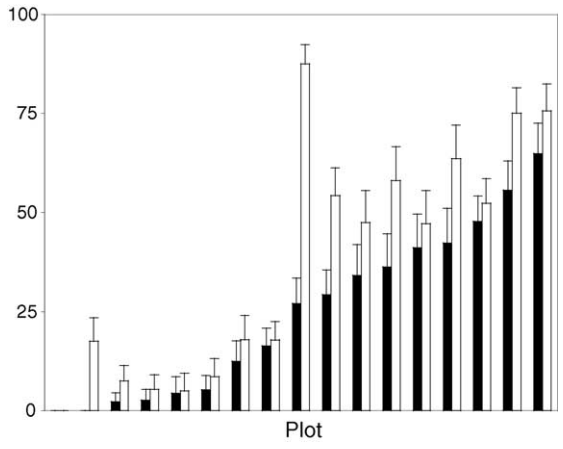

Fig. 1. Sudden oak death impact on *Q. agrifolia* (bleeding and symptomatic dead trees) as a percentage of the total number of trees in each plot (±S.E.), for 2000 (closed bars) and 2003 (open bars).

3.2. *Q. agrifolia and Q. kelloggii*

These oak species are considered to be closely related and hybridize readily. Their responses to *P. ramorum* infection appear to be very similar, although the numbers of *Q. kelloggii* in this study are much lower than *Q. agrifolia*. The results for *Q. kelloggii* are reported separately, but are included in the same sections as those for *Q. agrifolia*.

3.2.1. *Bleeding and dead trees*

At the start of the study in March 2000, there were 731 *Q. agrifolia* with stems greater than 5 cm dbh, of which 668 were alive and 167 (25.0%) were bleeding (Table 2). Bleeding trees constituted similar proportions of the living *Q. agrifolia* in each of the next three years, amounting to 23.6% in March 2003. The nearly static proportion of bleeding trees for the four dates obscures the true impact of sudden oak death in these plots. In March 2000, 5.8% of the trees were dead with evidence of bleeding, with the cumulative total rising to 17.4% by 2003 (Table 2). The numbers of dead trees without evidence of bleeding increased

Table 2
Percentages of *Q. agrifolia* in the asymptomatic, bleeding, dead with bleeding, and dead without bleeding (asymptomatic) categories in March of each year[a]

Year	*N* of Trees	Asymptomatic	Bleeding	Dead with bleeding	Dead without bleeding
2000	731	68.5	25.0	5.8	2.7
2001	731	64.8	25.1	10.3	3.2
2002	677	63.4	23.4	13.7	3.6
2003	677	60.1	23.6	17.4	3.8

[a] The percentages for live bleeding trees are based only on the total numbers of living trees. Dead tree percentages are based on total numbers of trees. Totals for 2002 are lower, due to removal of 18 hazardous (bleeding) trees in Plot C-1 and 36 fire-damaged trees in plot M-7.

much more slowly, from 2.7% in 2000 to 3.8% in 2003 (Table 2).

In March 2000, there were 53 *Q. kelloggii* in the plots, 15.7% of which were bleeding and 3.8% of which were dead with evidence of bleeding. In March 2003, 25% of trees were bleeding and 9.4%, all of which exhibited prior bleeding, had died.

3.2.2. Beetle colonization of bleeding trees

There was a statistically significant ($p < 0.0001$) association of bleeding *Q. agrifolia* stems with scolytid beetle colonization in each of the four years analyzed (Table 3). Of the 167 *Q. agrifolia* that were bleeding in March 2000, 52.7% were colonized or showed distinct evidence of previous beetle activity and a similarly close association was maintained for the following three years (Table 3).

Scolytid beetles had colonized 62.5% of bleeding *Q. kelloggii* in 2000 and 50% by 2003. For both dates, the association of beetles with bleeding trees was statistically significant by *G*-tests ($p < 0.0001$).

Table 3
Association of bark and ambrosia beetles with bleeding *Q. agrifolia* ($n = 668$ alive in 2000)[a]

Year	2000	2001	2002	2003
Total bleeding (n)	167	157	131	127
Bleeding with beetles (%)	52.7	58.0	56.5	42.5
LogLikelihood	134.6	150.5	113.81	76.84
R^2	0.501	0.57	0.503	0.424
Likelihood ratio (G statistic)	268.29	301.03	227.6	153.67
$P > G$	<0.0001	<0.0001	<0.0001	<0.0001

[a] Trees colonized at earlier date were included in subsequent counts even if no current activity was observed. Total numbers of trees are lower after 2001 due to fire and tree removal. LogLikelihood values are from contingency analysis of the association of beetles with bleeding trees.

Scolytid beetles colonized all the *Q. agrifolia* that died after 2000 while they were still living (with green foliage). The emergence of *H. thouarsianum* sporophores consistently followed beetle colonization.

3.2.3. Fate of same-symptom cohorts

For the trees that were alive (asymptomatic and symptomatic) in 2000, the status of a tree in 2000 was a statistically significant predictor of survival ($p < 0.0001$) for both Cox PH and Weibull survival models, whereas differences between China Camp State Park and MMWD were not significant. Even though the site effect was not significant, it was included in the models as an explanatory variable because the two sites differ in climate and species composition. Tree dbh from year 2000 was dropped from the survival models because this effect was not significant and the change in the AIC was minimal.

Fig. 2 depicts the survival probability estimated with Cox PH and Weibull regression models for China Camp State Park. The graph for MMWD is not shown because both sites have nearly identical graphs. The 50% probability of surviving longer than the median time is drawn for each category for the Weibull model. From these graphs, it becomes evident that both models are needed to evaluate the data for asymptomatic and bleeding trees, because the median survival time for both categories extends further than the three years of data that are available.

In the initially asymptomatic cohort of 468 trees, an individual was predicted to have a median survival time of 29.5 years (S.E. = 8.4) in China Camp State Park and 31.8 years (S.E. = 9.3) in MMWD (Table 4 and Fig. 2a). Both sites were pooled for the following results. After one year, 4.5% of the asymptomatic trees had developed bleeding; by 2003, this had increased to 11.3% (Table 5(a)), at which time 2.1% of this cohort was

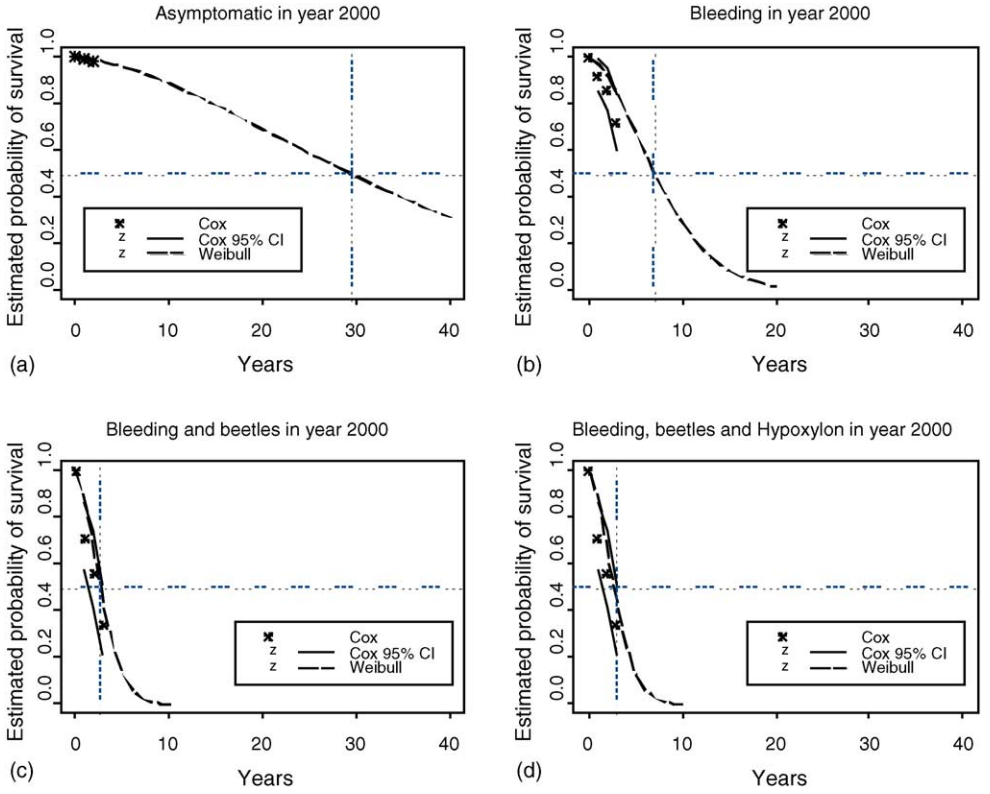

Fig. 2. Survival probabilities for *Q. agrifolia* cohorts in China Camp State Park, that were (a) asymptomatic, (b) bleeding, (c) bleeding with beetles, and (d) bleeding with beetles and *H. thouarsianum*, estimated using Cox PH (asterisks) and Weibull (dashed lines) regression models. The dashed vertical lines show where the probability of survival is 50%.

dead. By March 2001, beetles had colonized seven (31.8%) of the 21 bleeding trees that were initially asymptomatic. Through March 2003, 16 (30.2%) of these trees (including those that subsequently died) had been colonized. Beetles were associated with only 0.6 and 0.7% of the asymptomatic trees in 2000 and 2003, respectively (Table 5(b)). These trees showed evidence of declining health, including sporophores of decay fungi, but were not bleeding. After three years, eight (36.4%) of the trees that first developed bleeding in 2001 and that had been colonized by beetles also exhibited *H. thouarsianum* sporophores. Sporophores of *H. thouarsianum* were observed on six (1.3%) of the asymptomatic trees in this cohort in 2000, which was the greatest number observed in any year. The greatest number of asymptomatic trees colonized by beetles in any year was six (0.9%) (Table 5(b)).

In March 2000, there were 66 bleeding trees that had not been colonized by beetles (Table 5(c)). The Weibull model predicted a median survival time of a bleeding tree in China Camp State Park of seven years (S.E. = 1.2) and median survival time in MMWD of 7.6 years (S.E. = 1.6) (Table 4 and Fig. 2b). For both sites, after one year, beetles had colonized 23 (35%) of

Table 4
Q. agrifolia median survival time in years (±S.E.) from the Weibull fitted model

Status in year 2000	Asymptomatic	Bleeding	Bleeding and beetles	Bleeding and beetles and *H. thouarsianum*
Site CCSP	29.46 (8.40)	7.01 (1.21)	2.60 (0.32)	2.95 (0.31)
Site MMWD	31.75 (9.31)	7.56 (1.60)	2.80 (0.51)	3.18 (0.59)

Table 5
Cumulative changes in cohorts of *Q. agrifolia* with different initial symptom status[a]

Year	Asymptomatic (*n*)	Total bleeding[b]	Bleeding and beetles	Bleeding and beetles and *H. thouarsianum*	Dead with bleeding	Dead without bleeding
(a) Asymptomatic cohort (*n* = 468)						
2000	468	0	0	0	0	0
2001	444	21 (4.5%)	6	1	0	3
2002	430	30 (6.4%)	4	6	3	5
2003	405	53 (11.3%)	8	6	2	8

Year	Beetles only	*H. thouarsianum* only	*H. thouarsianum* and beetles
(b) Trees in the asymptomatic cohort that exhibited *H. thouarsianum* sporophores and beetles in the absence of bleeding			
2000	2	5	1
2001	0	4	1
2002	4	2	2
2003	1	2	2

Year	Bleeding only (*n*)	Bleeding and beetles	Bleeding and beetles and *H. thouarsianum*	Bleeding and *H. thouarsianum* only	Total dead	Dead with beetles
(c) Bleeding only cohort (*n* = 66)						
2000	66	0	0	0	0	0
2001	38	8	11	5	4 (6.1%)	4
2002	34	9	12	2	8 (12.1%)	7
2003	34	6	11	0	15 (22.7%)	14

Year	Bleeding and beetles only	Bleeding and beetles and *H. thouarsianum*	Dead
(d) Bleeding plus beetles cohort (*n* = 83)			
2000	37 (49.7%)	46 (55.4%)	0
2001	7 (11.9%)	52 (88.1%)	24 (28.9%)
2002	2 (4.8%)	40 (95.2%)	41 (49.4%)
2003	2 (9.1%)	20 (90.9%)	61 (73.5%)

[a] Trees that were fire-damaged or removed during the study were not included in these analyses.
[b] Total bleeding as a percentage of the initial cohort.

the live bleeding trees, four of which had died (Table 5(c)). Sporophores of *H. thouarsianum* had developed in 16 (58%) of these beetle-colonized trees (including four that died by 2001). By 2003, 22.7% of the initial cohort had died. After three years, beetles had colonized 17 (33%) of the surviving trees, and 11 (65%) of these also exhibited *H. thouarsianum* sporophores. The only bleeding tree that died without prior colonization by beetles broke due to structural weakness.

In March 2000, beetles had colonized 49.7% of the bleeding trees. Sporophores of *H. thouarsianum* were present on 55.4% of these beetle-colonized trees (Table 5(d)). The Weibull model predicted a median survival time of a beetle-colonized bleeding tree of 2.6 (S.E. = 0.3) years in China Camp State Park and 2.8 (S.E. = 0.5) years in MMWD (Table 4 and Fig. 2c). After one year, only 8.4% of the starting cohort did not have *H. thouarsianum* sporophores, and 28.9% of the trees were dead. By March 2003, only two trees were still free of *H. thouarsianum* sporophores, and 73.5% of the initial cohort was dead.

Weibull survival analysis of the cohort of beetle-colonized, bleeding trees that exhibited *H. thouarsianum* showed no differences in predicted survival compared with the trees without evidence of this fungus (Fig. 2d). For the 46 *Q. agrifolia* that had *H. thouarsianum* sporophores on the stems in 2000, 23.9% were still alive in 2003, compared with 29.7% survival of the *H. thouarsianum*-free trees. Every tree in this cohort that died by 2003 exhibited *H. thouarsianum* sporophores on the stem prior to death. In 2000, 37 *Q. agrifolia* were bleeding and colonized by beetles, but lacked sporophores of *H. thouarsianum*. After three years, only two trees (5.4%) of the starting cohort were still alive without signs of the fungus, whereas nine

(24.3%) of the living and 26 (70%) of the dead trees had developed these fruiting bodies.

The survival curves for the three cohorts (Fig. 3) clearly show that bleeding, and subsequent beetle colonization, were associated with progressive decreases in survival.

3.2.4. Relationship between tree diameter and bleeding status

For the 668 living *Q. agrifolia* in 2000, the mean diameter of all trees was 32.5 ± 0.6 cm. The mean dia–meter of the bleeding trees ($n = 149$), 37.6 ± 1.3 cm, was significantly greater than that of asymptomatic trees ($n = 469$), 29.6 ± 0.7 cm ($t_{1,660} = 5.39$, $p < 0.0001$). By 2003, the mean diameter of the 127 bleeding trees, 36.9 ± 1.7 cm, was still significantly greater than that of the 405 asymptomatic trees, 29.2 ± 0.7 cm ($t_{1,531} = 4.76$, $p < 0.001$). In 2000, the mean diameter of dead bleeding trees ($n = 42$), 38.2 ± 2.1 cm, was also significantly greater than dead, non-bleeding trees ($n = 20$), 27.4 ± 2.3 cm ($t_{1,60} = 3.48$, $p < 0.001$). Through 2003, the mean diameter of the 118 bleeding *Q. agrifolia* that died was 38.7 ± 1.4 cm, compared with that of the 26 dead and asymptomatic trees, 27.2 ± 2.2 cm ($t_{1,143} = 3.84$, $p = 0.0002$).

The diameters of bleeding trees were also positively correlated with beetle colonization. For *Q. agrifolia* in 2000, logistic regression of beetle status (present/absent) on log-transformed stem diameters of bleeding trees was significant ($\chi^2 = 41.1$, 1 DF, $p < 0.0001$).

For the trees that were asymptomatic in year 2000, a health failure analysis was performed using the Weibull regression model. Here survival time is defined as the time that a tree remains asymptomatic and failure is defined as the tree becoming symptomatic. Tree dbh measured in 2000 was a statistically significant predictor ($p = 0.002$) of health failure. Fig. 4a shows the modeled relationship between

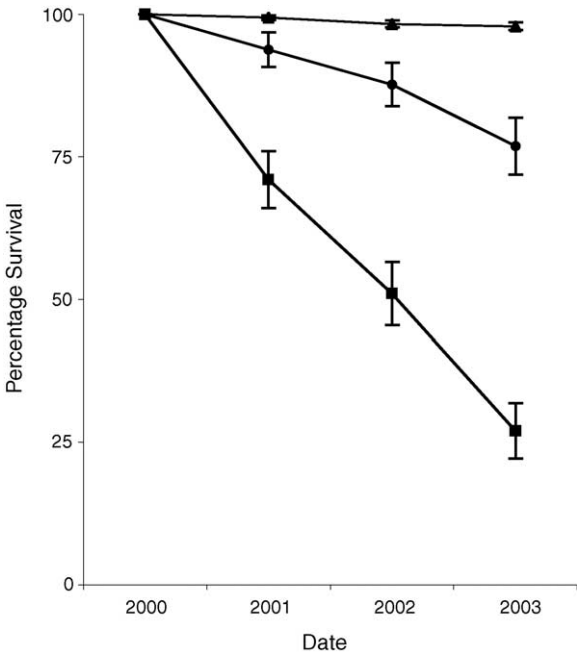

Fig. 3. Survival of the three *Q. agrifolia* cohorts: asymptomatic (triangles), bleeding only (circles), and bleeding with beetle colonization (squares), after 1, 2, and 3 years (±S.E.).

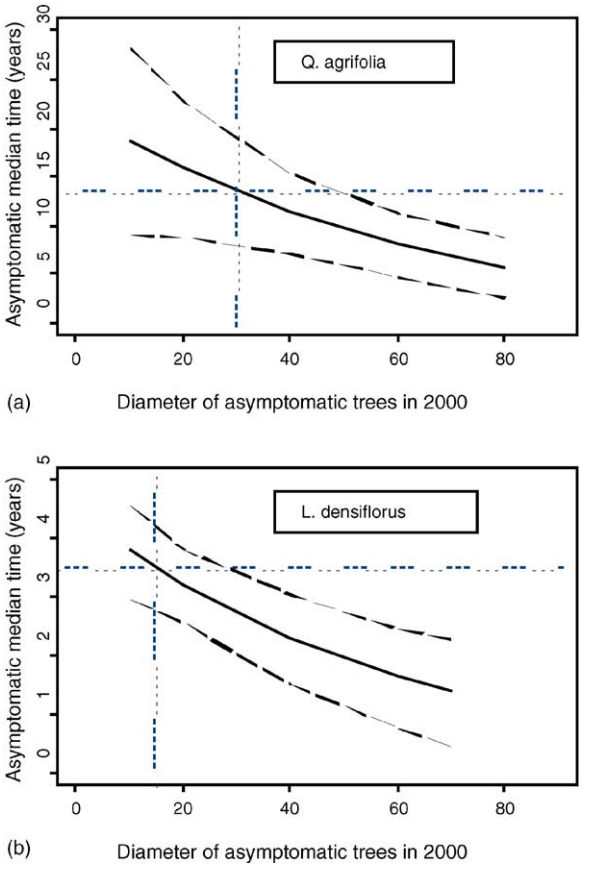

Fig. 4. Relationship of median survival time and tree dbh for the asymptomatic trees in year 2000. Graph a shows *Q. agrifolia* and graph b shows *L. densiflorus*. The vertical dashed lines represent the mean dbh for each species.

Table 6
Health analysis of *Q. agrifolia*, showing the median asymptomatic time in years (±S.E.) as a function of stem dbh (cm)

	dbh in 2000				
	dbh = 10	dbh = 20	dbh = 40	dbh = 60	dbh = 80
Site 1; CCSP	18.74 (4.78)	15.86 (3.47)	11.35 (2.04)	8.13 (1.64)	5.82 (1.54)
Site 2; MMWD	18.44 (5.08)	15.61 (3.72)	11.17 (2.17)	8.00 (1.65)	5.73 (1.52)

Q. agrifolia mean stem diameter and time a tree remained healthy. Larger trees were more likely to develop bleeding during the three years covered in the analysis (Table 6).

The mean diameter of the 51 *Q. kelloggii* that were alive in 2000 was 37.7 ± 2.1 cm. The mean diameter of the bleeding trees ($n = 8$), 50.8 ± 2.2 cm, was significantly greater than the asymptomatic trees ($n = 43$), 35.3 ± 2.2 cm, ($t_{1,50} = 2.89$, $p = 0.0055$). This relationship was also significant in 2003.

3.2.5. Structural failure of *Q. agrifolia*

Through March 2003, 12.2% (89 trees) of the *Q. agrifolia* that were standing in March 2000 had failed on the main stem, within 0.3 to 2 m of the soil line. Clear evidence of bleeding was observed on 84% of the failed trees, both living and dead. Only 7.7% of the living trees and 10% of the dead trees that failed were asymptomatic. Ambrosia beetle tunnels that predated the stem failure were present at the point of breakage in 34 (94.4%) of the 36 bleeding trees that failed with a green canopy and in 41 (91.1%) of the bleeding trees that failed after dying. Both beetle tunnels and sporophores of *H. thouarsianum* were present on 88.2% of the failed living trees. Failures of both living and dead trees rose markedly, from five through March 2001, to 35 through March 2002, and to 89 through March 2003.

3.3. *L. densiflorus*

3.3.1. Bleeding and dead trees

At the start of the study in March 2000, there were 181 living and dead *L. densiflorus*. Stem bleeding was present in 39.0% of these trees, 8.3% were dead with bleeding, and 1.1% were dead and asymptomatic (Table 7). By 2003, 62.4% of the trees were bleeding, 22.2% were dead with bleeding, and 3.0% were dead without bleeding. The combined proportions of bleeding and dead trees, on a plot basis, varied from 21 to 61% in 2000 and increased in every plot by 2003 (Fig. 5).

3.3.2. Bleeding and beetle colonization

In 2000, scolytid beetles had colonized 9.4% of the living *L. densiflorus* that were also bleeding. This percentage increased to 12.8% in 2003. The association of bleeding trees with beetles was statistically significant for each of these years (*G*-tests, $p < 0.01$ for each year).

3.3.3. Fate of same-symptom cohorts

For the trees that were alive (asymptomatic and symptomatic) in year 2000, the initial status of a tree was a statistically significant predictor of survival ($p < 0.0001$) for both Cox PH and Weibull survival models (Fig. 6). Tree dbh for year 2000 was dropped

Table 7
Percentages of asymptomatic, bleeding, and dead *L. densiflorus*[a]

Year	*N* of trees	Asymptomatic	Bleeding	Dead with bleeding	Dead with no bleeding
2000	181	55.3	39.0	8.3	1.1
2001	181	42.5	51.6	11.1	1.1
2002	167	32.3	58.5	19.8	2.4
2003	167	28.1	62.4	22.2	3.0

[a] For bleeding trees, percentages are based on total living trees. Dead tree percentages are based on total numbers of trees. For 2002 and 2003, the population size is lower due to removal of burned trees from the totals.

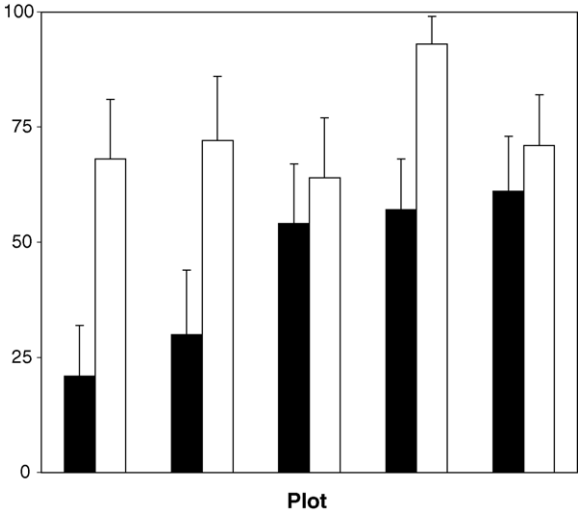

Fig. 5. Sudden oak death impact on *L. densiflorus* (bleeding and symptomatic dead trees) as a percentage of the total number of trees in each plot (±S.E.), for 2000 (closed bars) and 2003 (open bars).

from the survival models because this effect was not significant and the change in AIC was minimal.

In March 2000, 93 *L. densiflorus* were asymptomatic. After one year, 21.5% of these trees were bleeding and 2.2% were dead with evidence of bleeding (Table 8(a)). By March 2003, the total number of bleeding trees had increased to 38 (40.9% of the starting cohort), five (13.2%) of which had also been colonized by beetles, and eight (8.6%) were dead with bleeding. Weibull regression predicted a median survival time of 12.6 years (S.E. = 3.8) for a tree that was asymptomatic in 2000 (Table 9).

In 2000, 57 *L. densiflorus* were bleeding. By 2003, 14 (24.6%) of these trees were dead (Table 8(b)). Weibull regression predicted a median survival time of 8.7 years (S.E. = 2.3) for a tree that was bleeding in 2000 (Table 9). In 2000, beetles had colonized six (10.5%) of the bleeding *L. densiflorus*; four of these trees were dead by 2003. Of the 51 bleeding trees that were beetle-free in 2000, 10 (19.6%) had died by 2003

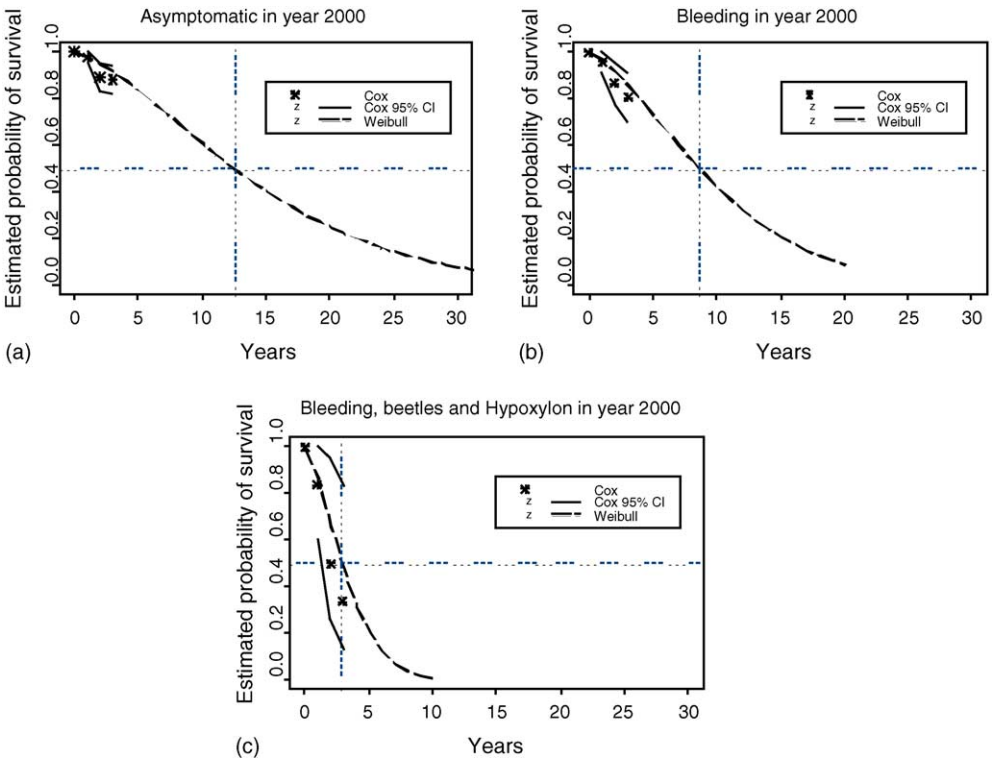

Fig. 6. Survival probabilities for *L. densiflorus* cohorts in Marin Municipal Water District that were (a) asymptomatic, (b) bleeding, and (c) bleeding with beetles (including trees with *H. thouarsianum*), estimated using Cox PH (asterisks) and Weibull (dashed lines) regression models. The dashed vertical lines denote the time to 50% survival probability.

Table 8
L. densiflorus cohort summary

Year	Asymptomatic	Total bleeding	Bleeding and beetles	Bleeding and *Hypoxylon*	Bleeding and Beetles and *Hypoxylon*	Dead with bleeding	Dead with no bleeding
(a) Asymptomatic cohort ($n = 93$)[a]							
2000	93	0	0	0	0	0	0
2001	71	20 (21.5%)	0	0	0	2	0
2002	54	29 (34.9%)	2	0	0	7	3
2003	44	38 (40.9%)	5	0	0	8	3

Year	Total bleeding	Bleeding and beetles	Bleeding and *Hypoxylon*	Bleeding and Beetles and *Hypoxylon*	Dead
(b) Bleeding Cohort, including those with beetles in 2000 ($n = 57$)					
2000	57	5	1	1	0
2001	54	6	1	3	3 (5.3%)
2002	51	5	0	3	6 (10.5%)
2003	43	4	0	3	14 (24.6%)
(c) Bleeding cohort, without beetles in 2000. This is a subset of the bleeding cohort ($n = 51$)					
2000	51	0	1	0	0
2001	49	3	1	1	2 (3.9%)
2002	46	3	0	2	5 (9.8%)
2003	41	2	0	3	10 (19.6%)

[a] Total bleeding as a percentage of the initial cohort.

(Table 8(c)); beetles had colonized half of these trees while they were still alive. Four of the six initially beetle-free trees that were colonized after 2000 had died by 2003. The Weibull regression model predicted that a beetle-colonized bleeding tree in 2000 had a median survival time of 2.9 years (S.E. = 1.0) (Table 9).

From the survival curves shown in Fig. 7, it is clear that the presence of bleeding cankers is strongly associated with decreased survival.

3.3.4. Relationship between *L. densiflorus* stem diameter and bleeding status

The mean diameter of all 181 *L. densiflorus* in the plots at the start of the study in 2000 was 16.1 ± 0.9 cm. Removal of fire-damaged trees reduced the mean diameter for all living stems ($n = 150$) to 15.8 ± 0.9 cm. Although initially not different, by 2003 the mean diameter of the bleeding trees ($n = 79$) was significantly larger, 16.4 ± 1.1 cm, than the asymptomatic trees ($n = 46$) 12.0 ± 1.6 cm ($t_{1,124} = 2.62$, $p = 0.0009$).

Table 9
L. densiflorus median survival time in years (±S.E.) from Weibull fitted model

Status in year 2000	
Asymptomatic	12.57 (3.82)
Bleeding only	8.66 (2.31)
Bleeding and beetles or bleeding and beetles and *H. thouarsianum*	2.85 (0.97)

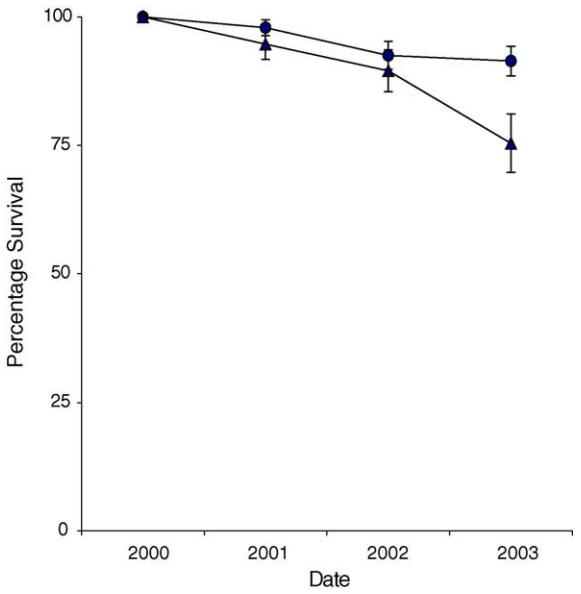

Fig. 7. Survival of asymptomatic (circles) and bleeding (triangles) *L. densiflorus* cohorts after 1, 2, and 3 years (±S.E.).

Table 10
Health analysis of *L. densiflorus*, showing the median asymptomatic time in years (±S.E.) as a function of dbh (cm)

dbh in year 2000				
dbh = 10	dbh = 20	dbh = 40	dbh = 60	dbh = 70
3.80 (0.41)	3.21 (0.32)	2.30 (0.39)	1.64 (1.80)	1.39 (0.47)

For the trees that were asymptomatic in 2000, a health failure analysis performed using the Weibull regression model found that dbh was a significant predictor ($p < 0.0064$) of future susceptibility to bleeding (Fig. 4b). For a tree with dbh of 40 cm, the mean for the population, the model predicted with a 50% probability that a tree would remain asymptomatic (median healthy time) for 2.3 years (S.E. = 0.4) (Table 10). For a tree with dbh of 10 cm, a tree was predicted to remain asymptomatic for 3.8 years (S.E. = 0.4).

4. Discussion

Each of the three tree hosts that are most consistently killed by *P. ramorum* in California, *Q. agrifolia*, *Q. kelloggii*, and *L. densiflorus*, followed a consistent sequence of signs and symptoms following the development of bleeding. Bleeding was the first visible symptom of sudden oak death in both oak species, followed by ambrosia beetle colonization, then emergence of *H. thouarsianum* sporophores. In *Q. agrifolia* and *Q. kelloggii*, the joint occurrence of scolytid beetles and *H. thouarsianum* preceded tree death, except for a few cases in which structural failure occurred before the foliage died. The sequence of symptoms and signs in *L. densiflorus* was generally similar to that of the oaks, although foliar symptoms (flagging and death of branch tips and individual leaves that resemble dieback caused by other biotic and abiotic agents) often appeared before bleeding, beetle colonization was less consistent, and *H. thouarsianum* sporophores only developed infrequently before death of a tree. The numbers of asymptomatic trees that developed bleeding and the numbers of symptomatic trees that died from 2000 to 2003 increased for all three species.

Survival analyses of same-symptom cohorts confirmed that sudden oak death is a progressive disease in *Q. agrifolia* and *L. densiflorus*. Decreased survival was strongly correlated in both species with beetle attacks on bleeding trees, and with subsequent emergence of *H. thouarsianum* sporophores on *Q. agrifolia*. Both Weibull and Cox PH survival analyses showed that, although the presence of this endophytic fungus was strongly associated with prior beetle attacks, survival did not decrease in trees that showed evidence of both organisms. This suggests that beetle colonization is the better predictor of mortality. These analyses also estimated very similar survival times for both species, approximately seven to nine years for bleeding and under three years for beetle-infested trees (Tables 4 and 9, Figs. 2 and 6). Once a tree has been infected by *P. ramorum*, the processes leading to death may be broadly similar in both species.

The Weibull model makes assumptions about the underlying distribution of the data. Results are thus influenced by the choice of parameters. The Cox proportional hazard model is semi-parametric, with fewer assumptions, but cannot extrapolate trends beyond the data set that is used. Considering these caveats, the models produce survival estimates that are in close agreement. The tendency for larger diameter trees to become symptomatic should lead to a positive correlation between dbh and mortality. Although the Weibull health analysis model found a significant relationship between stem dbh and development of the bleeding symptom for both *Q. agrifolia* and *L. densiflorus*, dbh was not a statistically significant factor in the survival analyses. This is likely due to the limited number of asymptomatic trees (the basis for the health analysis) that died during the study and to the fact that the trees in all other cohorts were symptomatic.

After three years, mortality in the initially asymptomatic *Q. agrifolia* cohort was only 0.4% for trees that developed the bleeding symptom (Table 5(a)). It is apparent that the mortality rate from this disease is a product of additional factors. Beetle colonization was associated with dramatically shortened survival in infected trees. In the initially bleeding *Q. agrifolia* cohort, mortality was preceded in all but one tree by scolytid beetle colonization of the bleeding area and development of *H. thouarsianum* sporophores in the vicinity of the bleeding cankers. The trees that were dead in 2000 with evidence of bleeding, beetles, and *H. thouarsianum* sporophores are assumed to have gone through the same sequence.

The high mortality in the bleeding plus beetles cohort, as well as the development of *H. thouarsianum* sporophores on all but seven trees after one year, clearly indicates that most of these trees had been infected for more than three years when the study began (Table 5(d)). The consistent presence of insects and fungi in *Q. agrifolia* infected with *P. ramorum* illustrates the difficulty in isolating mortality caused by the pathogen from mortality influenced by the secondary organisms that respond to infected oaks. Although it is probable that infected trees would develop secondary fungal infections and decline in the absence of beetle colonization, this study shows that once beetles colonized a tree, its probability of survival declined rapidly.

The damage caused by *P. ramorum* cankers alone seems unlikely to lead to structural failure of mature trees, since this pathogen has not been reported to penetrate deeper than approximately 3 cm into the sapwood (Rizzo et al., 2002). The presence of associated organisms, principally ambrosia beetles and *H. thouarsianum*, was highly correlated with both symptomatic living and dead *Q. agrifolia* that failed. The point where green and bleeding trees snapped off typically exhibited extensive ambrosia beetle tunnels to a depth of 10 cm and greater into the sapwood, and in numbers sufficient to plausibly cause structural weakening. Beetle penetration of the outer bark breaches the primary defense of a tree against pathogens and tunneling into the outer sapwood disrupts water conduction. Thus, these beetles can create avenues for entry of decay fungi, as reported for a number of other pathogen-tree-beetle interactions (Anderson and Hoffard, 1978; Carpenter et al., 1988; Hijii et al., 1991; Kessler, 1974) and may also facilitate the development of endophytic fungi, such as *H. thouarsianum*. In other hardwood species, decay located on the main stem can lead to breakage of living trees. *Hypoxylon mammatum* infection can lead to structural failure in living *Populus* spp. (Manion and Griffin, 1986). The joint activity of the fungi, *Nectria* spp., beech scale (*Cryptococcus fagisuga*), and ambrosia beetles (*Xyloterus domesticum*), in European beech bark disease can result in beech snap, the structural failure of mature *Fagus sylvatica* trees (Speight, 1981).

The localized response of native ambrosia and bark beetles to *P. ramorum*-induced bleeding cankers on native oaks constitutes a shift in their host selection behavior. The principal species involved are two ambrosia beetles, the oak ambrosia beetle (*Monarthrum scutellare*) and the minor oak ambrosia beetle (*M. dentigerum*), and the western oak bark beetle (*Pseudopityophthorus pubipennis*), which colonize fresh dead limbs of living trees, trees that are freshly killed or broken, or trees severely weakened by disease (Chamberlain, 1958; Furniss and Carolin, 1977; Wood, 1982). Ambrosia beetles inoculate their tunnels and brood chambers with co-evolved fungi that serve as food for both adults and developing larvae (Beaver, 1989). Prior invasion of the sapwood by mycelia of decay fungi should prevent successful ambrosia beetle colonization through competitive exclusion of the ambrosia fungi. Because these beetles' fungi are at a competitive disadvantage, selective pressures favor beetle colonization of host trees with sound, undecayed wood. A tree that is in decline caused by decay fungi probably does not provide suitable host material for ambrosia beetles. In a stand of *Q. agrifolia* infected with *P. ramorum*, those trees that are severely weakened, as indicated by sparse and pale foliage, are assumed to provide suitable host material for these beetles. These trees may undergo mass beetle attack at this stage. However, bleeding cankers are the only visual evidence of *P. ramorum* infection in *Q. agrifolia* prior to beetle attack. On a freshly broken (non-infected) *Q. agrifolia*, the characteristic colonization pattern is not localized on the lower bole, but is distributed relatively uniformly over the entire tree (McPherson, unpublished observation). Where bleeding cankers are present on a living tree, beetles initially colonize only the bark that overlies the cankers. The cues to which these insects respond are unknown, but the close proximity of their tunneling activity to bleeding sites on the bark suggests that infected subcortical tissues may produce volatiles that signal the presence of suitable host material.

The criteria that are reliably used to evaluate symptomatic *Q. agrifolia* are not always adequate to detect infected *L. densiflorus*. Since the relationship between foliar symptoms and stem cankers is not known for the latter species, evaluations based on bleeding alone are likely to underestimate the true extent of infection in a stand. This conclusion is derived from both the Weibull survival analyses

(Tables 4 and 9) and mortality in asymptomatic cohorts (Tables 5 and 8) for both species. Although the mortality recorded for the initially asymptomatic cohorts was proportionally higher for *L. densiflorus* than *Q. agrifolia* after three years, both the predicted and observed mortality of the bleeding and beetle-infested cohorts were similar in these species. The Weibull analysis predicted that the survival time of a tree in the asymptomatic *Q. agrifolia* cohort was more than twice that of an asymptomatic *L. densiflorus* (Figs. 2 and 5). This difference could be due to a greater susceptibility of *L. densiflorus* to *P. ramorum* infection. Very high infection and mortality levels have been observed in this species in Marin and Monterey County forests, and in a number of areas, a majority of the mature trees have been infected or killed. It is also probable that infected *L. densiflorus* are undercounted, because infected trees may not all have bleeding cankers at the early stages of the disease.

The high structural failure rate of living *Q. agrifolia* with bleeding cankers (5.8% of the total stems alive in 2000) indicates that *P. ramorum* infection has altered the rate of failure in this species. The role played by beetles in tree failure cannot be separated from that of *H. thouarsianum*, as indicated by the presence of their sporophores. However, because beetle tunneling precedes the development of *H. thouarsianum* sporophores, beetles are the more consistent and reliable predictor of failure in living *Q. agrifolia* infected by *P. ramorum*.

The finding that larger diameter trees are more likely to develop sudden oak death suggests that mean stem diameters have been reduced since the epidemic started. The association of tunneling beetles with larger diameter bleeding *Q. agrifolia* also will decrease the mean diameter in the population, since the survival analysis showed that once beetles colonize a bleeding tree, its expected survival is significantly decreased. Taken together, these results suggest that as *P. ramorum* continues to infect new trees, the size distribution of both *Q. agrifolia* and *Q. kelloggii* will shift downward. This process may lead to recruitment of new seedling cohorts in these overstory-dominated forests, as the forest canopy opens. Whereas infection of oak foliage is rare, the foliage of *L. densiflorus* is much more susceptible to *P. ramorum* infection (Davidson et al., 2002). In closed canopy forests, *L. densiflorus* saplings and seedlings can be abundant in the understory. Since *P. ramorum* is a lethal pathogen for *L. densiflorus* in all age classes, including seedlings, saplings, and mature trees (Rizzo et al., 2002), this species is threatened as a major component of these forests.

The infection process is not yet understood for bark cankers. The tendency for *P. ramorum* cankers to be primarily confined to the bark and cambium (Rizzo et al., 2002) could indicate that bark quality affects infections in oaks and tanoaks. In the absence of any documented mechanism for infection through the bark, the possible role of insects in the spread of *P. ramorum*, though speculative, should be considered. A number of characteristics potentially predispose insects to serve as vectors of tree pathogens (Leach, 1940). Because none of the beetle species associated with *P. ramorum*-infected oaks are reported to attack healthy trees, these insects are not likely candidates as vectors. Attempts to isolate *P. ramorum* from ambrosia and bark beetles emerged from infected logs have not been successful (unpublished data). Beech bark disease of *Fagus grandifolia* in eastern North America results from the interaction of an introduced scale insect (*Cryptococcus fagisuga*) and both introduced and native *Nectria* spp., and is more likely to occur in larger diameter trees (Houston, 1975). The elevated nitrogen content of the bark in larger trees leads to selective feeding by these insects (Wargo, 1988). Where a source of inoculum is abundant, unrecognized wounding by insects that selectively feed on larger diameter trees may serve as infection courts for *P. ramorum*. If infection is a result of a randomly distributed process, the greater surface area and more complex bark surface structure of larger trees may increase the probability of infection.

The persistence of asymptomatic host trees that are surrounded by bleeding and dead trees may indicate disease resistance within these stands (Dodd et al., 2005). Assuming that *P. ramorum* was recently introduced into these forests, some proportion of the population would have become infected, but as fewer of the resistant individuals develop infections in later years, the number of newly symptomatic *Q. agrifolia* should stabilize or decline. Evidence for variation in resistance of *Q. agrifolia* to *P. ramorum* is suggested by studies in which mechanical inoculations produced cankers that varied considerably in size and

in frequency of disease development among trees (McPherson et al., unpublished data; Rizzo et al., 2002).

Acknowledgements

We appreciate the field assistance of Dan Stark, Gabriela Ritok-Owens, Jake Schweitzer, Danny Fry, and Brandon Collins. Patrick Robards (China Camp State Park), George Gray (California State Parks), and Mike Swezy (MMWD) provided access to protected properties and other assistance. Nadir Erbilgin provided a helpful review of the paper. Funding has been provided by the University of California Division of Agriculture and Natural Resources, USDA-Forest Service, Pacific Southwest Research Station, and the University of California Statewide Integrated Pest Management Program for Exotic/Invasive Pests and Diseases.

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