Evidence for the role of synchronicity between host phenology and pathogen activity in the distribution of sudden oak death canker disease

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Summary

• Variations in synchronicity between colonization rate by the pathogen and host phenology may account for unexplained spatial distribution of canker disease. The hypothesis that synchronous pathogenicity and host development are necessary for incidence of sudden oak death disease was tested by correlating seasonal variations in host cambial phenology and response to inoculation with *Phytophthora ramorum*.

• Response to infection was estimated by inoculating branch cuttings from coast live oak (*Quercus agrifolia*) trees at nine dates through a full annual cycle in 2003–2004. Host phenology was estimated from measurements of bud burst and cambial activity in spring 2006.

• Lesions were largest in the spring soon after the cambium resumed activity. A moderate genetic component to lesion size was detected. Variation among trees in date of largest lesions correlated with variation in timing of bud burst and cambial phenology.

• The data support the hypothesis that active host cambial tissue is a necessary requisite for successful infection with the pathogen that causes sudden oak death canker disease. Genetic variation in host phenology will buffer coast live oak against epidemics of this disease.

Key words: exotic pathogen, inoculation, resistance, *Phytophthora ramorum*, *Quercus agrifolia*, spatial distribution, synchronicity.

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Introduction

Biological invasions, as a result of increased international trade and transportation, are one of the single most important threats to the health of native ecosystems. Among these invasive species, exotic pathogens easily pass undetected during an initial lag phase. Although in the majority of cases exotic pathogens do not cause significant damage (Jones & Baker, 2007), there are many examples in which they have caused enormous damage to plant populations (Desprez-Loustau *et al.*, 2007). Initial survival of the invasive pathogen depends on a susceptible host(s) and a disease-conducive environment. Subsequent spread will be mediated by ecological and biological interactions between the hosts and the pathogen (Burdon & Jarosz, 1988; Gilbert, 2002). Unlike native pathogens that may modify structure and diversity of plant communities slowly over time, invasive pathogens can result in rapid and severe transformation of plant communities as they are brought into contact with naïve populations of a previously unexposed host (Burdon *et al.*, 2006). When the new host is a large keystone species, structural change in the plant community can be far-reaching because of the cascading effects on associated animal and plant life which may lead to increased or decreased diversity (Burdon *et al.*, 2006). Dramatic

examples of changes in plant community structure following disease introductions include loss of diversity in native jarrah (*Eucalyptus marginata*) communities in south-western Australia after the introduction of *Phytophthora cinnamomi* (Hardham, 2005), increased diversity with the emergence of new plant communities composed of co-dominant shrubs and trees to replace chestnut (*Castanea dentata*) in eastern North American forests after the introduction of Asian *Cryphonectria parasitica* (Stephenson 1986) and the replacement of elm (*Ulmus* spp.) by shade-tolerant species in eastern North American forests following invasion by *Ophiostoma ulmi* (Parker & Leopold, 1983). Although tree mortality may provide increased nesting sites for birds and animals (Franklin *et al.*, 1987), loss of heavy seed-producing species may have adverse effects on diversity of wildlife (Castello *et al.*, 1995; Monahan & Koenig, 2006).

Symptoms of disease in natural forests and woodlands commonly are highly variable through time and space. This variability results from interactions among biotic and environmental layers that are inherently heterogeneous. Such interactions present formidable challenges and opportunities to understand and manage the dynamics of disease spread. Landscape pathology attempts to integrate host and pathogen systems and to develop spatially explicit models that help in the prediction of disease spread and dynamics (Holdenrieder *et al.*, 2004; Ostfeld *et al.*, 2005; Hamelin, 2006). In addition to spatial interactions, the physiological dependence between host and pathogen that results from phenological changes in the host also determines the development of disease (Biere & Honders, 1996, Kennelly *et al.*, 2005; Blachinsky *et al.*, 2006). Native pathogens evolve synchronicity with host phenological cycles, particularly for diseases of ephemeral organs such as flowers and fruits (Ngugi & Scherm, 2006). However, incidence of disease caused by introduced pathogens will be variable depending on chance matching between host and pathogen phenology. Such variation could explain in part the patchy distribution of disease that is often observed in natural forests and woodlands. For pathogens causing canker diseases, the availability of newly differentiating vascular tissues in the host may be a prerequisite for disease progression. If the timing of sporulation events is seasonal and it coincides with the period of resumption of cambial activity in the host, infection is likely. However, hosts with late cambial activity would escape infection during the main sporulation event. This is likely to be particularly true of splash-dispersed pathogens in Mediterranean environments, where peak sporulation events are rainfall and temperature limited. Variations in host phenology could therefore partly explain patchiness in host mortality; when the pathogen peaks, the host must be available.

Sudden oak death (SOD), an emerging disease caused by an introduced pathogen, has provided the opportunity to evaluate the importance of synchronicity between host and pathogen in terms of the incidence and spatial distribution of disease. Mortality of oaks (*Quercus* spp. Sect. Lobatae) and tanoak (*Lithocarpus densiflorus*), resulting from infection by *Phytophthora ramorum* (Rizzo *et al.*, 2002), is threatening to transform the coastal woodlands of central and northern California. The spatial distribution of disease on coast live oak (*Quercus agrifolia*) is uneven (Kelly & Meentemeyer, 2002), but the causes are still poorly understood. Sporulation of the pathogen is at a maximum between the months of December and May when climate is cool and humid (Davidson *et al.*, 2005). Coast live oak is a keystone species of California's coastal woodlands, providing habitat for a wide range of insects, birds and mammals. It forms a vegetation type in central and southern California that is primarily single species (78% of basal area) and is relatively dense compared with other oak species in California (Waddell & Barrett, 2005).

Our earlier work indicated significant heritable variation in lesion size on branch cuttings of coast live oak in response to inoculation with *P. ramorum* (Dodd *et al.*, 2005). Here, we extend this earlier work to ask whether variations in host cambial phenology could contribute to the patchiness of mortality of coast live oak and whether genetic resistance can be detected through shifts in susceptibility when a stand has been exposed to an epidemic of the disease. We used three approaches. First, we followed the response of branch cuttings to inoculation throughout a full annual cycle in 2003–2004 to test for seasonal variation in responsiveness. We estimated clonal repeatability to determine whether variations in response had a genetic base. Secondly, we sampled bud and cambial tissue of the same trees in spring 2006 to determine variations in phenology of bud burst and cambial activity. We recognize that it would have been preferable to have phenological data for the same year as inoculations. However, variations among trees are likely to remain constant across seasons because of high heritability of phenological traits in trees (Morgenstern, 1996; Rehfeldt *et al.*, 1999). Over the relatively small spatial scale of our sampled trees, local climatic variations from one year to the next are unlikely to be important. We then investigated whether individuals with later resumption of shoot and cambial activity also produced maximum lesions at a later date. Finally, we anticipated that stands having suffered high mortality would have been purged of the more susceptible genotypes. We hypothesized that this could be detected as smaller average lesion sizes and lower variability in lesion size. For this, we compared two neighbouring stands that contrasted in levels of prior mortality as a result of SOD disease.

Materials and Methods

Sample locations

Coast live oak trees were selected from two sites within the China Camp State Park, Marin County, California, USA. This is a mixed evergreen coastal woodland that includes *Quercus agrifolia* Née, *Lithocarpus densiflorus* (Hook. & Arn.)

Rehd., *Umbellularia californica* ((Hook. & Arn.) Nutt.), and *Arbutus menziesii* (Pursh) as dominants, *Quercus kelloggii* (Newb.) and *Quercus lobata* (Née) as scattered individuals and *Quercus wislizeni* (A.DC.) and *Pseudotsuga menziesii* ((Mirbel) Franco) at higher elevations. Coast live oak mortality from SOD has been severe in this Park. Cumulative mortality from 2000 to 2003 was 21.2%, with considerable variation among plots (McPherson *et al.*, 2005). Here, we selected one site, where significant SOD-related mortality had thinned the stand by > 40% (Miwok), and a second site at which mortality was < 1% and infection ranged from 1 to 5% (Chicken Coop Island). The former site is in continuous woodland and the latter site is on a rise, isolated from the adjacent forest by grassland and salt marsh. Both sites face north towards the San Pablo Bay and are separated by *c.* 0.4 km. Elevation at Miwok ranged from *c.* 15 to 40 m and at Chicken Coop Island from *c.* 8 to 20 m. Trees, with a minimum diameter at breast height of 25 cm and with a broad crown, were selected at random for inoculation studies; 17 trees were selected at the Miwok site and 15 at the Chicken Coop Island site.

Climatic data for 2003 and 2004 were obtained from a nearby weather station at Point San Pedro, which is approx. 3.7 km from the sample sites and is at sea level and facing east towards the San Pablo Bay (http://www.cimis.water.ca.gov/cimis/monthlyReport.do).

Plant material

Several branches were removed from the outer, lower crown of trees following protocols in Dodd *et al.* (2005) at nine dates from March 2003 until March 2004 (24 March 2003, 21 April 2003, 20 May 2003, 24 June 2003, 14 August 2003, 1 October 2003, 25 November 2003, 18 February 2004, and 24 March 2004; referred to here as inoculations 1–9, respectively) to furnish cuttings for inoculation. Branches were cut and their ends were wrapped in moist tissue during transport and then placed in buckets of water in a 12 C room, to avoid desiccation. Cuttings, approx. 1 cm in diameter at the base and *c.* 25–40 cm in length, were excised from the harvested branches. Branch cuttings, with foliage, were stood in jars of sterilized water covered with Parafilm (American National Can, Chicago, IL, USA), with the stems protruded through the Parafilm into the water.

Pathogen inoculum

Isolate Pr102 (ATCC MYA-2949) of *P. ramorum* was recovered from the stem canker of *Q. agrifolia* in Sonoma County, California, in 2001. Isolate Pr-102 was found to be moderately pathogenic on oak seedlings (Hüberli *et al.*, 2006) and it belongs to the most common clonal genotype of *P. ramorum*, present almost ubiquitously in California forests (Ivors *et al.*, 2004). Inoculum discs of 5 mm diameter were cut with a sterile cork borer from the margin of 14-d-old cultures grown at 20 C on vegetable-8 juice (Campbell Soup Company) agar (Hüberli *et al.*, 1997) with omission of β-sitosterol and $CaCO_3$. The isolates are stored in the culture collection of D. Rizzo, University of California, Davis, CA, USA.

Inoculation and test conditions

A sterile cork borer was used to remove a 6-mm-diameter disc of bark *c.* 11 cm from the base of the branch cutting, well above its level of immersion in water. An inoculum disc was placed mycelium-side-down on the exposed cambial surface, the plug of bark was replaced and the inoculated area was wrapped with Parafilm and then silver Nashua® tape (Tyco Adhesives, Lexington, MA, USA) for protection. For each individual tree, four or five replicate branch cuttings were inoculated. In addition, one branch cutting from each individual was inoculated with a noncolonized agar plug. A single control per tree was used because our earlier studies have shown that negative controls behave similarly and so controls across parent trees can be pooled. Branch cuttings were randomly placed on a glasshouse bench under natural day-lengths and ambient temperatures, with automatic misting. Supplemental water was given to the jars as needed. The average daily temperatures for the nine inoculations ranged from 14.6 to 20.5 C.

Harvest and measurements

Branch cuttings were harvested after 21 d. Diameters of branch cuttings were measured at the site of inoculation. The outer bark around the site of inoculation was carefully scraped with a scalpel in order to expose the entire lesion. Vertical distances of infection above and below the site of inoculation and the maximum distance around the girth of the segment were recorded. Small pieces of tissue cut from the margins of the longitudinal lesion and the site of inoculation were plated onto pimaricin-ampicillin-rifampicin-pentachloronitrobenzene (PCNB) agar (P_{10}ARP) containing one quarter the amount of PCNB, a selective medium for *Phytophthora* spp. (Erwin & Ribeiro, 1996), to confirm the presence of *P. ramorum*.

Phenological observations

Branch cuttings, equivalent in size to those used for inoculations, were taken from the 32 trees at weekly intervals from 21 February to 30 May 2006 to examine phenological differences among individuals. Phenological observations were only made in the spring months to follow resumption of activity. For each tree, the date of bud flushing (emergence of leaves from within the bud scales for at least 50% of buds) was recorded. Stem samples were removed for microscopical analysis of width of the cambium and differentiating vascular tissue.

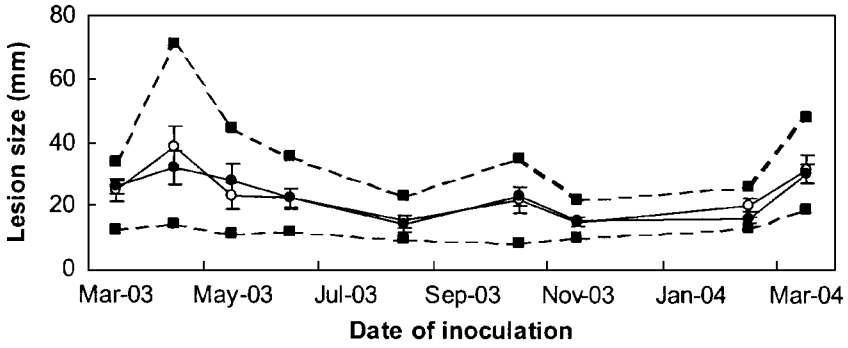

Fig. 1 Size of lesions at different inoculation dates in coast live oak (*Quercus agrifolia*) after inoculation with *Phytophthora ramorum*. Open circles, Chicken Coop site; closed circles, Miwok site. Overall maximum and minimum lesion sizes are shown as dotted lines with closed rectangles. Standard errors are shown as vertical bars.

Data analysis

We found no correlation between lesion length and diameter of the branch cutting. Lesion sizes were common-logarithm-transformed for all data analyses, except for mean standardized lesion sizes. We tested for significant lesions attributable to *P. ramorum*, by running one-sided *t*-tests for each tree at each inoculation date, comparing the lesion sizes of all replicates of a tree at an inoculation date with the lesion size of controls of all trees at that date.

Variance in lesion size was partitioned (1) among individuals within sites and (2) between sites using a mixed model analysis of variance, with sites as a fixed effect and trees within sites as a random effect. Analyses of variance were carried out on data for each inoculation date and on the mean of the standardized lesion sizes at each of the inoculation dates. Untransformed lesion sizes at each inoculation date were divided by their mean to obtain standardized lesion size. We did this to prevent a few inoculation dates, at which large lesions were produced, from dominating the overall estimates. All analyses of variance were performed using the GLM procedure in SAS (SAS Institute, Cary, NC, USA).

Clonal repeatability (r) was estimated at each inoculation date by estimating the variance components using the branch cutting replicates as clonal replicates. Variance components were estimated by the VARCOMP procedure in SAS, using the restricted maximum likelihood method. Standard errors of clonal repeatability were estimated as the root of intraclass variances following the approximate formula (Swiger *et al.*, 1964)

$$\text{SE } r \approx \sqrt{[2(N-1)(1-r)^2[1+(k-1)t]^2/k^2(N-s)(s-1)]}$$

(N, the total number of observations; s, the number of groups; $k = [N-(\Sigma n_i^2/N)]/(s-1)$; n_i, number of observations in group; r, the clonal repeatability.)

Results

As in our previous work, cuttings placed in hydroponic culture survived well on the glasshouse bench. New foliage was only produced on some cuttings in the spring inoculations. At 3 wk after inoculation, *P. ramorum* produced lesions that extended above and below the site of inoculation. *Phytophthora ramorum* was successfully re-isolated from all inoculated cuttings, but not from controls. One-tailed *t*-tests revealed only 24 cases (8.1%) in which lesions from inoculated plants were not significantly larger than lesions from controls. Of these, 10 were for the fourth inoculation date on 24 June 2003. The nonsignificant differences from controls were mostly scattered among individuals, with only one tree having nonsignificant differences at three or more of the inoculation dates.

Seasonal variation in lesion size

Mean lesion size reached a maximum during the spring inoculation (inoculation 2) on 21 April 2003, decreased through the summer months and reached a second peak during the autumn inoculation (inoculation 6) on 1 October 2003 (Fig. 1, Table 1). In 2004, mean lesion size increased over the two early spring inoculations, to reach a maximum at the second of these (inoculation 9) on 24 March 2004. Mean lesion sizes followed seasonal climate variations; the spring maxima in lesion size occurred when mean weekly air and soil temperatures at the nearby weather station at Point San Pedro had risen above 12 C and evapotranspiration measured above grassland was *c*. 4 mm (Fig. 2). As temperatures of the air and soil and evapotranspiration increased, average lesion sizes decreased. The smaller peak in lesion size in October 2003 occurred when air and soil temperatures began to decline and evapotranspiration had once again reached *c*. 4 mm.

Variation among individuals

Variation in lesion size among individuals within sites was significant at each inoculation date and explained from 15 to 43% of variance and 46% of variance of the standardized mean over all dates (Table 1). Variance components for sites and trees within sites were greatest in the spring months when lesion sizes were large and the among-individual component was also high in the autumn (6th inoculation

Table 1 Mean lesion sizes by site and inoculation date and probabilities of site effects and tree effects on lesion size in coast live oak (*Quercus agrifolia*)

	Mean lesion size (mm)				
	Chicken Coop	Miwok	*P* of site effect	*P* of tree effect (site)	Varcomp % tree (site)
Inoculation 1	24.7	26.4	0.23	0.0006	16.0
Inoculation 2	38.3	33.1	0.002	< 0.0001	43.2
Inoculation 3	27.3	29.0	0.11	< 0.0001	26.4
Inoculation 4	22.4	22.8	0.42	0.0004	21.4
Inoculation 5	15.3	14.3	0.15	0.003	16.5
Inoculation 6	21.9	23.1	0.19	< 0.0001	32.4
Inoculation 7	15.0	15.1	0.62	0.007	14.7
Inoculation 8	20.4	15.7	< 0.0001	0.003	16.3
Inoculation 9	34.7	30.2	0.004	0.0001	25.1
Standardized mean	1.04	0.97	0.025	< 0.0001	46.0

Effects on lesion length were estimated from analysis of variance and variance components using the restricted maximum likelihood method with trees within sites as random effects and sites as fixed effects.

Table 2 Lesion size repeatability estimates (*r*) obtained as the ratio of the among-tree variance component to the total phenotypic variance component, 95% confidence interval (CI) and error per cent of mean estimate (in parentheses) at each of nine inoculation dates and across inoculation dates in coast live oak (*Quercus agrifolia*)

	Chicken Coop site		Miwok site	
	r	CI(*r*)	*r*	CI(*r*)
Inoculation 1	0.42	±0.11 (14.1)	0.16	±0.08 (32.5)
Inoculation 2	0.46	±0.11 (12.6)	0.43	±011 (12.2)
Inoculation 3	0.19	±0.10 (28.7)	0.35	±0.11 (14.1)
Inoculation 4	0.14	±0.10 (35.4)	0.27	±0.11 (17.2)
Inoculation 5	0.22	±0.07 (25.8)	0.26	±0.11 (19.1)
Inoculation 6	0.36	±0.11 (16.4)	0.30	±0.11 (18.2)
Inoculation 7	0.17	±0.09 (31.7)	0.22	±0.10 (21.5)
Inoculation 8	0.21	±0.11 (26.8)	0.26	±0.11 (19.6)
Inoculation 9	0.34	±0.11 (17.3)	0.18	±0.11 (29.3)
Temporal repeatability				
All inoculations	0.16	±0.005 (17.7)	0.18	±0.006 (12.9)
Inoculations 2, 6 and 9	0.30	±0.01 (11.5)	0.21	±0.008 (15.5)

Table 3 Pearson correlation coefficients computed on standardized lesion sizes of the same coast live oak (*Quercus agrifolia*) trees at two spring inoculations (inoculation 2, spring 2003; inoculation 9, spring 2004) and an autumn inoculation (inoculation 6, autumn 2003)

	Inoculation 2	Inoculation 6
All trees		
Inoculation 6	0.063 (0.42)	
Inoculation 9	0.231 (0.003)	0.039 (0.63)
Chicken Coop		
Inoculation 6	0.097 (0.41)	
Inoculation 9	0.270 (0.02)	0.054 (0.64)
Miwok		
Inoculation 6	0.047 (0.67)	
Inoculation 9	0.118 (0.27)	0.029 (0.79)

Probabilities are shown in parentheses.

date), when a secondary peak in lesion size was detected. Among-tree ranges in lesion size, shown as upper and lower bounds in Fig. 1, closely followed seasonal variations in mean lesion size. The range in lesion sizes among trees varied from a factor of 2 for inoculation 8 to a factor of *c*. 4 for inoculations 2, 3 and 6.

Clonal repeatability

Repeatability estimates for each inoculation date indicate significant variation among trees in lesion size after inoculation (Table 2). Estimates ranged from 0.14 to 0.46 and larger estimates tended to be at those inoculation dates that produced the largest lesion sizes. At the Chicken Coop site, lesion size and repeatability were significantly correlated ($r_{\text{Chicken Coop}} = 0.73$, $P = 0.04$), but at Miwok the correlation was not significant ($r_{\text{Miwok}} = 0.51$, $P = 0.20$). Standard errors ranged from 12 to 35% of the repeatability estimates.

The temporal estimates of repeatability were also significantly different from zero (Table 2). Removing inoculation dates at which lesion sizes were relatively small greatly improved the estimates. Pearson correlations of lesion size at the three dates of largest lesions revealed significant correlation between the two spring inoculations (inoculations 2 and 9), but not with the autumn inoculation (Table 3).

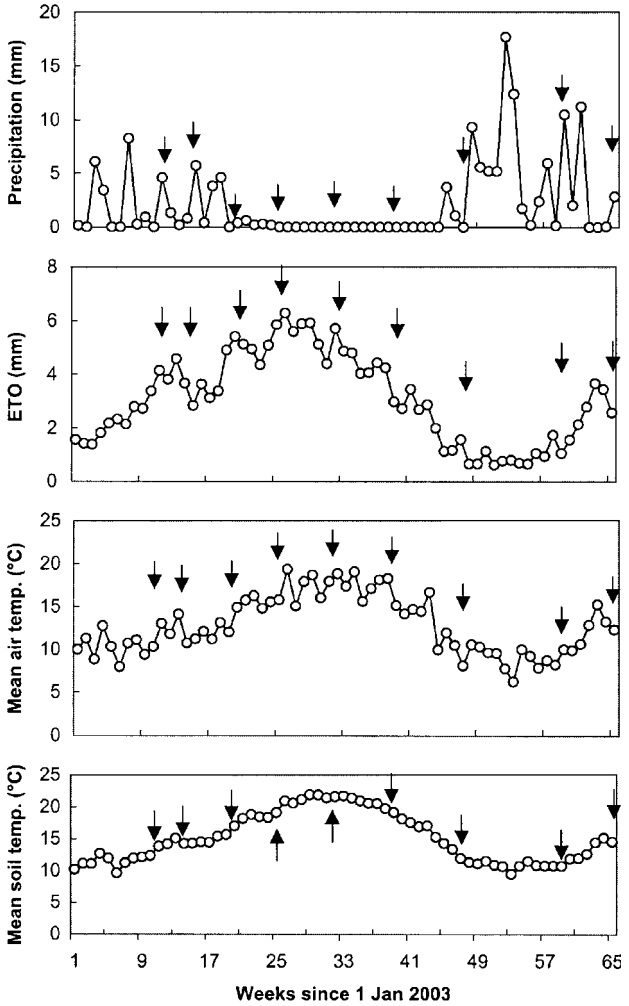

Fig. 2 Plots of weekly mean data of climatic variables taken from the Point San Pedro climate station (http://www.cimis.water.ca.gov/cimis/monthlyReport.do). ETO, evapotranspiration measured over grassland. Arrows indicate inoculation dates in this study.

Synchrony in seasonal trends among individuals and correlations with shoot activity

Most trees produced their largest lesions in the second inoculation on 21 April 2003. However, some individuals produced a peak lesion size earlier or later than others, indicating variation in the timing of their response to inoculation (Table 4). We hypothesized that individual response to inoculation was correlated with physiological activity in the cambium, which would vary from tree to tree according to differences in phenology. As our inoculation dates were at approx. 1-month intervals in the spring of 2003, we cannot detect at a fine scale the date at which each individual would have produced its maximum lesion size. We measured the phenology of the same trees in the spring of 2006. Earliest bud burst occurred at the Miwok site, where all sampled trees had burst buds by 15 March (Table 4). At Chicken Coop, earliest bud burst in the sample trees was a week later than at Miwok and extended until 29 March. The onset of cambial activity (measured as swelling of cambial initials and enlargement of the cambial zone (cambial initial plus differentiating xylem and phloem)) began in one tree at the end of February (Table 4). Approximately 1 month elapsed between the earliest and latest trees resuming activity. Onset of cambial activity lagged behind bud burst by *c.* 2 wk, but variation among trees in the two phenological traits was strongly correlated (Pearson's correlation coefficient $r_{\text{Chicken Coop}} = 0.84$, $P < 0.0001$; $r_{\text{Miwok}} = 0.79$, $P = 0.0005$). The earliest mature vessels were detected in some trees in late April. The onset of cambial activity preceded the date of maximum lesion size by *c.* 5 wk, which was about the time that the first mature vessels were produced. Variation in onset of cambial activity and timing of maximum lesion size were significantly correlated at the two sites (Pearson's correlation coefficient $r_{\text{Chicken Coop}} = 0.56$, $P = 0.03$; $r_{\text{Miwok}} = 0.79$, $P = 0.0001$).

Table 4 Numbers of coast live oak (*Quercus agrifolia*) trees with maximum lesion size and cumulative numbers of trees with bud flushing and cambial activity at different dates

	Chicken Coop site	Miwok site
Maximum lesion size		
24 March	0	3
21 April	12	10
20 May	2	4
24 June	1	0
Bud flushing		
21 February	0	11.8
1 March	13.3	47.1
8 March	60.0	76.5
15 March	80.0	100.0
22 March	93.3	
29 March	100.0	
Cambial activity		
1 March	0	0.6
8 March	0	35.3
15 March	33.3	58.8
22 March	60.0	76.5
29 March	86.7	100.0
5 April	100.0	

Lesion sizes were measured in 2003, and bud and cambial activity were measured on the same trees in 2006. Bud flushing was estimated as 50%, or more, of buds open.

Site differences in lesion size

Mean standardized lesion size was significantly larger (6.2%) in cuttings from the Chicken Coop site (Table 1). This was attributable to statistically significant site effects for spring inoculations, when lesions were large (inoculations 2 and 9). For these inoculations, 75 and 63%, respectively, of trees in the upper quartile of lesion size were from the Chicken Coop

site. Although mean repeatability over all inoculation dates did not vary between the two sites, it was greater at Chicken Coop for inoculation dates with the largest lesion sizes (Table 2). This was supported by significant Pearson correlations between spring inoculations (inoculations 2 and 9) at Chicken Coop, but not at Miwok (Table 3).

Coefficients of variation of mean lesion size were calculated for each harvest date either from the standard deviations of replicate samples per tree averaged over trees, or from the standard deviations of replicate trees. Paired sample *t*-tests across harvest dates found significantly greater coefficients of variation at the Chicken Coop site for both estimates (replicates per tree: $t_{\text{Chicken Coop – Miwok}} = 3.57$; $P > t = 0.004$; replicate trees: $t_{\text{Chicken Coop – Miwok}} = 2.34$; $P > t = 0.024$).

Discussion

Spatial variability of disease in natural forests and woodlands may result from environmental heterogeneity, biotic factors, including the presence of hosts upon which the pathogen can sporulate, and transmission pathways that facilitate spore dispersal. Diversity of host genotypes may also contribute directly through resistance genes, or indirectly through variations in host phenology that lead to escape for some individuals that are not in synchrony with the pathogen. We set out to test whether variation in the timing of host response to inoculation was associated with phenological variation in shoot and cambial activity that might be important in determining the degree and spatial distribution of mortality of coast live oak caused by the introduced pathogen *P. ramorum.*

Correlation between host phenology and pathogen colonization

The size of lesions produced after inoculation of branch cuttings varied over an annual cycle, with maxima in the spring months and a secondary peak in the autumn of 2003. During the summer months, inoculations produced a lesion from which the pathogen could be recovered and lesions were larger than wound responses produced by controls, but lesions were small. Therefore, the pathogen was capable of spreading through vascular tissue at all times during the year, but its progress was most rapid in the cool, wet spring months and was slowest in the hot, dry summer months.

Mediterranean woody plants may display very different patterns of cambial activity that appear to be determined in part by phylogenetic constraints and by adaptations that afford avoidance of summer drought stress (Avila *et al.*, 1975; Mooney *et al.*, 1977). Coast live oak began cambial activity *c.* 2 wk after bud burst in the spring and mature vessels were produced *c.* 4 wk later. Although phenology was not measured in the same year as the inoculations, heritable variation in bud burst and cambial activity is generally very high in woody species (Morgenstern, 1996). Year-to-year variations in climate may promote or delay phenology, but are not likely to change the relative order of re-activation among individuals over the small spatial scale of this study. Assuming similar dates of phenology in 2003 as those measured in 2006, the spring 2003 peak in lesion size in late April would have been after the cambium had resumed activity and at about the time of first mature vessel production. Variation in cambial activity and timing of maximum lesion size among trees sheds further light on the possible dependence of pathogen colonization on active cambium. As inoculations were carried out at approx. 1-month intervals and phenological measurements were taken weekly, the precision of the relationship between the traits is weakened. However, our data are consistent with a hypothesis that host tissue colonization by the pathogen is greatest when cambium is undergoing active divisions. Coast live oak is reported to cease activity by early July (Avila *et al.*, 1975), but activity of the cambial zone would decrease much earlier as cells go through their phases of maturation (Dodd & Fox, 1990). The timing of peak lesion size varied among trees over the first three inoculation dates in 2003 (Table 4). Variation in cambial phenology among trees was well correlated with variation in time of maximum lesion size among trees, providing further evidence for the role of an active cambium in growth of the pathogen.

Phytophthora species are usually thought of as colonizing phloem or cambial tissue (Erwin & Ribeiro, 1996) with only minor incursions into the xylem. However, recent evidence shows that *Phytophthora* can invade up to 2.5 mm into the xylem and that the vertical pattern of outbreaks in the phloem matches the course of vessels in which spores or mycelium may be carried (Brown & Brasier, 2007). Also, colonization of xylem by *P. ramorum* has been confirmed in *Rhododendron* (Parke, 2007). Our data showing a correlation between an active cambium and pathogen colonization suggest that the pathogen was growing in the cambium and phloem rather than in older differentiated xylem. Late season artificial inoculations have been reported to produce deeper lesions than in the spring (Garbelotto *et al.*, 2007). It would be interesting to know if the pathogen preferentially penetrates deeper into the xylem later in the season, where water is more abundant.

Synchronicity of host physiology and pathogen sporulation events

In the mixed evergreen forest, maximum infectious sporulation of *P. ramorum* occurs in the spring. Although the production of propagules may start as early as January, when significant rain events are recorded in California, infection of new hosts seems to be linked to warmer temperatures, such as those recorded in April and May (Davidson *et al.*, 2005). We suggest that an active cambium that may be necessary for maximum pathogen colonization coincides with the timing of reported high infectiousness of the pathogen (Davidson *et al.*, 2005). We believe such synchronous conditions may be one of the

underlying reasons for the SOD epidemic in coast live oak populations. The synchronicity of pathogen sporulation and host physiological function appears to be critical and could explain why some hosts that become physiologically active later in the spring could avoid infection. Resumption of shoot and cambial growth in the spring are known to be heritable traits in woody plants (Morgenstern, 1996; Rehfeldt *et al.*, 1999) and in coast live oak woodlands, trees are consistently early or late flushing (Koenig *et al.*, 1996). Our phenological data revealed differences of more than 3 wk between trees in time of flushing and of more than 1 month in time of cambial swelling. Variations in phenology of this order are not uncommon in oaks and have been shown to lead to differences in the incidence of foliar herbivores (Crawley & Akhteruzzaman, 1988). In coast live oak, late flushers could still have a relatively narrow cambial zone at the critical time when sporulation of the pathogen becomes limiting. As a result, these individuals would escape the main wave of sporulation. Oaks require a heavy load of inoculum for infection to occur, so a strict synchrony of cambial activity with the main sporulation events may be more important than in other susceptible taxa. Could this explain why the later flushing white oaks of Section *Quercus* (*Quercus garryana* (Dougl. ex Hook.) and *Quercus douglasii* (Hook. & Arn.)) do not show symptoms of disease where they are sympatric with coast live oak in natural woodlands of California? Susceptibility of white oaks has been detected on *Quercus robur* L. in the more humid conditions of south-western England (Brasier *et al.*, 2004) and in artificial inoculations of oaks of Sections *Cerris* and *Quercus* (Brasier *et al.*, 2005, Tooley & Kyde, 2007), suggesting that these species are not inherently resistant.

Heritable variation in response to inoculation

Tests of clonal repeatability confirmed that lesion size is a heritable trait, although environmental components were high. We are unable to separate out the environmental effects that are passed from mother tree to cuttings from the genetic component. Consequently, our estimates of clonal repeatability provide only an upper bound for broad sense heritability. All estimates were < 0.5, indicating that environmental effects were greater than genetic effects. Clonal repeatabilities were greater at inoculation dates that produced large lesion sizes and greater differences in lesion size among individuals. Whereas many trees maintained similar rankings in spring 2003 and spring 2004, rankings produced when lesion sizes were small were not consistent. This underlines the importance of carrying out assays of this type during the spring months when large lesions are produced.

One of the goals of the study was to determine whether variation in susceptibility among oaks plays a significant role during waves of the SOD epidemic. Our approach was to test whether susceptibility of survivors after an intense epidemic is lower than the average susceptibility of comparable local individuals yet to be exposed to the epidemic. The site we selected, China Camp State Park, is ideal for this type of test as at the time of our study it encompassed both stands with the highest recorded level of infection and mortality as well as stands characterized by low levels of both. Average lesion sizes and variability of lesion size within and among trees were greater at Chicken Coop, the site with low levels of infection, than at the heavily impacted site at Miwok. Although other factors could account for these differences, the data suggest that we are detecting selection against more susceptible individuals at Miwok. If validated, this is promising as it suggests that sufficient genetic variation exists in natural populations of coast live oaks, because, even under intense disease epidemics, less susceptible individuals can survive that will regenerate the gaps left through mortality.

Among the 32 parent trees assayed here from two sites in a local woodland, mean lesion sizes varied by up to about four times. This level of variation was consistent with our previous results from a population study, and confirms the importance of variation among trees within a population that we reported earlier (Dodd *et al.*, 2005). Consequently, the genetic structure of oak populations exposed to SOD may be shifting, with selection favouring less susceptible individuals.

Future disease management

If our hypothesis that SOD canker disease depends on synchronicity between sporulation events and active cambial tissue is valid, a trend towards warmer temperatures predicted for coastal California is likely to exacerbate disease incidence through earlier phenology of hosts. Many reports of changes towards earlier flowering and leafing associated with climate change appear in the literature (Schwartz & Reiter, 2000; Khanduri *et al.*, 2008). Such a trend is likely to increase the infection window, and increasing temperatures will probably lead to increased plant stress and action by secondary pathogens. Genetic diversity is high within coast live oak stands (Dodd *et al.*, in press) and this will provide a buffer to the effects of disease on coast live oak. However, fragmentation of oak woodlands may lead to loss of genetic diversity (R. S. Dodd, unpublished data), so that management should be towards conservation of woodland size and connection corridors.

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References

Avila G, Aljaro ME, Araya S, Montenegro G, Kummerow J. 1975. The seasonal cambium activity of Chilean and California shrubs. *American Journal of Botany* **62**: 473–478.

Biere A, Honders SJ. 1996. Impact of flowering phenology of *Silene alba* and *S. dioca* on susceptibility to fungal infection and seed predation. *Oikos* **77**: 467–480.

Blachinsky D, Shtienberg D, Zamski E, Weinthal D, Manulis S. 2006. Effects of pear tree physiology on fire blight progression in perennial branches and on genetic expression of pathogenicity genes in *Erwinia amylovora*. *European Journal of Plant Pathology* **116**: 315–324.

Brasier C, Denman S, Brown A, Webber J. 2004. Sudden Oak Death (*Phytophthora ramorum*) discovered on trees in Europe. *Mycological Research* **108**: 1108–1110.

Brasier CM, Rose J, Kirk S, Denman S, Webber J. 2005. Comparative host range and aggressiveness of *Phytophthora ramorum* and *Phytophthora kernoviae* sp. nov. on North American and European trees. In: Frankel SJ, Shea PJ, Haverty MI, eds. *Proceedings of the Second Sudden Oak Death Science Symposium: The state of our knowledge, 2005, January 18–21, Monterey, CA*. Gen. Tech Rep. PSW-GTR-196. Albany, CA, USA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture, 109–111.

Brown AV, Brasier CM. 2007. Colonization of tree xylem by *Phytophthora ramorum*, *P. kernoviae* and other *Phytophthora* species. *Plant Pathology* **56**: 227–241.

Burdon JJ, Jarosz AM. 1988. The ecological genetics of plant-pathogen interactions in natural communities. *Philosophical Transactions of the Royal Society of London B* **321**: 349–363.

Burdon JJ, Thrall PH, Ericson L. 2006. The current and future dynamics of disease in plant communities. *Annual Review of Phytopathology* **44**: 19–39.

Castello JD, Leopold DJ, Smallidge PJ. 1995. Pathogens, patterns, and processes in forest ecosystems. *BioScience* **45**: 16–24.

Crawley MJ, Akhteruzzaman M. 1988. Individual variation in the phenology of oak trees and its consequences for herbivorous insects. *Functional Ecology* **2**: 409–415.

Davidson JM, Wickland AC, Patterson HA, Falk KR, Rizzo DM. 2005. Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. *Phytopathology* **95**: 587–596.

Desprez-Loustau M-L, Robin C, Buée M, Courtecuisse R, Garbaye J, Suffert F, Sache I, Rizzo DM. 2007. The fungal dimension of biological invasions. *Trends in Ecology and Evolution* **22**: 472–480.

Dodd RS, Afzal-Rafii Z, Mayer W. (in press). Molecular markers show how pollen and seed dispersal affect population genetic structure in coast live oak (*Quercus agrifolia* Née). In: Standiford RB, ed. *Proceedings of the Sixth Symposium on Oak Woodlands: Today's challenges, tomorrow's opportunities.* Albany, CA, USA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Dodd RS, Fox P. 1990. Kinetics of tracheid differentiation in Douglas-fir. *Annals of Botany* **65**: 649–657.

Dodd, RS, Hüberli D, Douhovnikoff V, Harnik TY, Afzal-Rafii Z, Garbelotto M. 2005. Is variation in susceptibility to *Phytophthora ramorum* correlated with population genetic structure in coast live oak (*Quercus agrifolia* Née)? *New Phytologist* **165**: 203–214.

Erwin DC, Ribeiro OK. 1996. *Phytophthora diseases worldwide*. St Paul, MN, USA: APS Press.

Franklin JF, Shugart HH, Harmon ME. 1987. Tree death as an ecological process. *BioScience* **37**: 550–556.

Garbelotto M, Schmidt DJ, Harnik TY. 2007. Phosphite injections and bark application of phosphite + Pentabark® control sudden oak death in coast live oak. *Arboriculture and Urban Forestry* **33**: 309–317.

Gilbert GS. 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

Hamelin RC. 2006. Molecular epidemiology of forest pathogens: from genes to landscape. *Canadian Journal of Plant Pathology* **28**: 167–181.

Hardham AR. 2005. *Phytophthora cinnamomi. Molecular Plant Pathology* **6**: 589–604.

Holdenrieder O, Pautasso M, Weisberg PJ, Lonsdale D. 2004. Tree diseases and landscape processes: the challenge of landscape pathology. *Trends in Ecology and Evolution* **19**: 446–452.

Hüberli D, Afzal-Rafii Z, Dodd RS, Douhovnikoff V, Harnik TY, Meshriy M, Miles L, Reuther K, Garbelotto M. 2006. Interactions of *Phytophthora ramorum* with two native Californian trees: bay laurel and coast live oak, from. In: Brasier C, Jung T, Oßwald W, eds. *Progress in research on phytophthora diseases of forest trees. Proceedings of the Third International IUFRO Working Party S07.02.09 Meeting in Freising, Germany, 11–18 September 2004*. Surrey, UK: Forest Research, Farnham, 79–83.

Hüberli D, Tommerup IC, Hardy GE St. J. 1997. The role of paragynous and amphigynous antheridia in sexual reproduction of *Phytophthora cinnamomi*. *Mycological Research* **101**: 1383–1388.

Ivors KI, Hayden KJ, Bonants PJM, Rizzo DM, Garbelotto M. 2004. AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. *Mycological Research* **108**: 378–392.

Jones DR, Baker RHA. 2007. Introductions of nonnative plant pathogens into Great Britain, 1970–2004. *Plant Pathology* **56**: 891–910.

Kelly NM, Meentemeyer RK. 2002. Landscape dynamics of the spread of sudden oak death, *Photogrammet. Engineering Remote Sensing* **68**: 1001–1009.

Kennelly MM, Gadoury DM, Wilcox WF, Magarey PA, Seem RC. 2005. Seasonal development of ontogenetic resistance to downy mildew in grape berries and rachises. *Phytopathology* **95**: 1445–1452.

Khanduri VP, Sharma CM, Singh SP. 2008. The effects of climate change on plant phenology. *Environmentalist* **28**: 143–147.

Koenig WD, Knops JMH, Carmen WJ, Stanback MT. Mumme RL. 1996. Acorn production by oaks in central coastal California: influence of weather at three levels. *Canadian Journal of Forest Research* **26**: 1677–1683.

McPherson BA, Mori SR, Wood DL, Storer AJ, Svihra P, Kelly NM, Standiford RB. 2005. Sudden oak death in California: Disease progression in oaks and tanoaks. *Forest Ecology and Management* **213**: 71–89.

Monahan WB, Koenig WD. 2006. Estimating the potential effects of sudden oak death on oak-dependent birds. *Biological Conservation* **127**: 146–157.

Mooney HA, Kummerow J, Johnson A, Parsons D, Keeley S, Hoffman A, Hays R, Gilberto J, Chu C. 1977. The producers – their resources and adaptive responses. In: Mooney HA, ed. *Convergent evolution in Chile and California – Mediterranean climate ecosystems*. Stroudsburg, PA, USA: Dowden, Hutchinson and Ross, 85–143.

Morgenstern EK. 1996. *Geographic variation in forest trees*. Vancouver, Canada: University of British Columbia Press.

Ngugi HK, Scherm H. 2006. Biology of flower infecting fungi. *Annual Review of Phytopathology* **44**: 261–282.

Ostfeld RS, Glass GE, Keeing F. 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends in Ecology and Evolution* **20**: 328–336.

Parke JL. 2007. Root and stem infection of rhododendron from potting medium infested with *Phytophthora ramorum*. *Plant Disease* **91**: 1265–1270.

Parker GR, Leopold DJ. 1983. Replacement of *Ulmus americana* L. in a mature east-central Indiana wood. *Bulletin of the Torrey Botanical Club* **110**: 482–488.

Rehfeldt GE, Ying CC, Spittlehouse D, Hamilton DA. 1999. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. *Ecological Monographs* **69**: 375–407.

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* **86**: 205–214.

Schwartz MD, Reiter BE. 2000. Changes in North America spring. *International Journal of Climatology* **20**: 929–932.

Stephenson SL. 1986. Changes in a former chestnut-dominated forest after a half century of succession. *American Midland Naturalist* **116**: 173–179.

Swiger LA, Harvey WR, Everson DO, Gregory KE. 1964. The variance of intraclass correlation involving groups with one observation. *Biometrics* **20**: 818–826.

Tooley PW, Kyde KL. 2007. Susceptibility of some eastern forest species to *Phytophthora ramorum*. *Plant Disease* **91**: 435–438.

Waddell KL, Barrett TM. 2005. *Oak woodlands and other hardwood forests of California, 1990s*. PNW-RB-245. Portland, OR, USA: US Department of Agriculture, Forest Service, Pacific Northwest Research Station.

