

Attraction of ambrosia and bark beetles to coast live oaks infected by *Phytophthora ramorum*

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- Abstract**
- 1 Sudden oak death is caused by the apparently introduced oomycete, *Phytophthora ramorum*. We investigated the role of bark and ambrosia beetles in disease progression in coast live oaks *Quercus agrifolia*.
 - 2 In two Marin County, California sites, 80 trees were inoculated in July 2002 with *P. ramorum* and 40 were wounded without inoculation. Half of the trees in each group were sprayed with the insecticide permethrin [cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-(3-phenoxyphenyl) methyl ester] to prevent ambrosia and bark beetle attacks, and then were sprayed twice per year thereafter. After each treatment, sticky traps were placed on only the permethrin-treated trees. Beetles were collected periodically in 2003.
 - 3 Inoculated trees accounted for 95% of all beetles trapped. The ambrosia beetles *Monarthrum scutellare* and *Xyleborinus saxeseni* and the western oak bark beetle *Pseudopityophthorus pubipennis* were the most abundant of the seven species trapped.
 - 4 Permethrin treatment delayed initiation of beetle attacks and significantly reduced the mean number of attacks per tree. Beetles did not attack any wounded or noncankered inoculated trees.
 - 5 Trees with larger cankers trapped more beetles early in the disease. Once permethrin lost effectiveness, the number of beetle entrance tunnels was a more reliable predictor of subsequent trap catch than was canker size.
 - 6 Beetles were initially attracted to *P. ramorum* cankers in response to kairomones generated in the host-pathogen interaction. After beetles attacked the permethrin-treated trees, aggregation pheromones most probably were the principal factor in beetle colonization behaviour.

Keywords Ambrosia and bark beetles, attraction, coast live oak, Coleoptera, host colonization, permethrin, *Phytophthora ramorum*, *Quercus agrifolia*, Scolytidae, Sudden oak death.

Introduction

Sudden oak death (SOD) disease is caused by an apparently introduced oomycete, *Phytophthora ramorum* (S. Werres & A.

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W. A. M. de Cock) (Werres *et al.*, 2001; Rizzo *et al.*, 2002). This pathogen has a broad host range in North America, infecting native plants in 14 families (Garbelotto *et al.*, 2003; RAPRA, 2007). Tanoaks *Lithocarpus densiflorus* (Hook. & Arn.) and red oaks in the subgenus *Erythrobalanus* are the species most consistently infected and killed in California. Distinct stem cankers develop on coast live oak *Quercus agrifolia* (Nee), black oak *Quercus kelloggii* (Newberry), Shreve oak *Quercus parvula* var. *shrevei* (C. H. Muller) Nixon and

tanoak. Canyon live oak *Quercus chrysolepis* (Liebm.), which is in the golden oak (*Protobalanus*) group, is susceptible to foliar infections but is rarely killed (Murphy & Rizzo, 2003). Tanoak mortality that was subsequently attributed to *P. ramorum* was first reported in Marin County, California, in 1994 and coast live oak mortality was observed in 1995 (Svihra, 1999a).

This pathogen is now established in coastal areas of California, from Monterey County to Humboldt County. Isolated infestations are currently found in Curry County, southwestern Oregon. In addition to established infestations in forested areas, a number of infected plants in a wide range of families have recently been found in nurseries throughout the western U.S.A. and Canada (Rizzo *et al.*, 2005). In Europe, the disease is limited primarily to nurseries, although infected mature oaks and other species have been found in sites adjacent to nurseries (Brasier *et al.*, 2004).

The earliest visible symptom of *P. ramorum* infection in coast live oaks is production of a viscous exudate from the bark overlying cankers, referred to as bleeding. After bleeding develops, a number of beetles (Coleoptera: Scolytidae), primarily the ambrosia beetles *Monarthrum scutellare* (LeConte) and *Monarthrum dentigerum* (LeConte), and the western oak bark beetle *Pseudopityophthorus pubipennis* (LeConte) (Coleoptera: Scolytidae) have been reported tunneling into the bark overlying bleeding cankers (Svihra, 1999a, b; McPherson *et al.*, 2000; McPherson *et al.*, 2005). Initially, beetles tunnel only within canker margins (McPherson *et al.*, 2005). However, at approximately the time the foliage begins to change colour (to pale green), these beetles colonize the remainder of the tree. Death of entire trees typically follows within a few months. Sporocarps of the saprotrophic fungus *Hypoxyton thouarsianum* (Leveille) Lloyd, typically develop on the bark of bleeding trees after beetle attacks (McPherson *et al.*, 2005).

Ambrosia beetles and western oak bark beetles commonly colonize highly stressed and recently killed trees, suppressed and dead branches, and stumps (Furniss & Carolin, 1977). In a 3-year study, between 43% and 58% of *P. ramorum*-infected bleeding coast live oaks had been colonized by ambrosia and bark beetles and more than 95% of the bleeding trees that died had been previously colonized by these beetles when the foliage was still green and apparently healthy (McPherson *et al.*, 2005). In the same study, Weibull survival models predicted a median survival of approximately 8 years for naturally-infected trees that had not been attacked by beetles, decreasing to 3 years for trees that had been colonized by beetles. Rarely, symptomatic trees have been observed that died without apparent beetle attacks.

Bleeding coast live oaks have a high rate of main stem failures (Swiecki & Bernhardt, 2003; McPherson *et al.*, 2005). Breakage in both living and dead trees occurs where ambrosia beetle tunnels are abundant (Fig. 1) (McPherson *et al.*, 2005). Application of permethrin to the bark of cut logs reduced beetle galleries by 75% and decreased the cross sectional area of fungal decay by 90% in *P. ramorum*-infected coast live oaks that had failed when the foliage was still green (Svihra & Kelly, 2004). Inhibition of ambrosia beetle tunneling resulted in decreased fungal decay of sapwood.



Figure 1 Structural failure of a living coast live oak tree in January 2002 followed tunneling by ambrosia beetles (tunnels are lined with black fungi) that were highly concentrated in the region of the *Phytophthora ramorum* canker. This tree was inoculated in July 2000 and showed no signs of decline prior to breakage.

The present study reports the responses of ambrosia and bark beetles to mature coast live oaks that were mechanically inoculated with *P. ramorum*. We quantified the attraction of ambrosia and bark beetles to cankered areas on infected trees and compared the trap catch with that from mock-inoculated trees. We tested the hypotheses that: (i) infected trees are more attractive to bark and ambrosia beetles than trees that are simply wounded; (ii) beetle attraction is influenced by canker size and by the presence of beetles tunneling in infected trees; and (iii) permethrin protects inoculated trees from bark and ambrosia beetle attacks.

Materials and methods

Study design, inoculation and insecticide application

Two sites were selected in protected coastal oak woodlands (Mayer & Laudenslayer, 1988) in Novato (33°22'58.570" N; -122°32'13.760" E) and San Rafael (37°59'39.701" N; -122°30'0.401" E) (referred to as the Nike site), Marin County, California. Abundant woody plants on these sites include bay laurel *Umbellularia californica* (Hook. & Arn.) Nutt., madrone *Arbutus menziesii* (Pursh.), toyon *Heteromeles arbutifolia* (Lindl.) M. Roemer and coast live oak. Naturally *P. ramorum*-infected coast live oaks are abundant in these sites. On each site, 60 asymptomatic coast live oaks, with a mean \pm SE diameter at breast height (DBH) of 30.6 \pm 0.97 cm in the Nike site and 31.7 \pm 0.87 cm in the Novato site, were randomly assigned to two treatments. Forty trees in each site were wounded, followed by inoculation with *P. ramorum* and 20 trees were wounded only (mock-inoculated). Inoculations generally followed the procedure described in Rizzo *et al.* (2002). A 2-cm diameter hole saw powered by a cordless drill was used to remove a plug of bark and phloem tissue, down to the sapwood. A section of *P. ramorum* culture on agar (approximately 5 \times 5 mm) was placed against the sapwood

surface and the bark plug was replaced. Aluminum foil was placed over the wound and the plug was held in place with tape for several weeks to minimize contamination and desiccation of cut tissue. Inoculation holes were drilled approximately 1.5 m above the soil line, the location depending on tree bark roughness, location of branches and ease of access. To mock-inoculate a tree (wounded only), a bark plug was drilled without insertion of an agar plug and the wound was covered as described above. Mock-inoculated trees were included to test for beetle attraction to the wounds in the absence of *P. ramorum* infection. Trees were inoculated on 23 and 27 July 2002 in San Rafael and on 25 and 27 July 2002 in Novato. In 2003, trees were evaluated at: day 275 (April), day 300 (May), day 333 (June), day 364 (July), day 450 (October), day 464 (November) and day 500 (December) after inoculation.

Insecticide application

Half of the trees in each treatment were randomly selected for insecticide application and sprayed to run-off using a backpack sprayer, to a stem height of 2.5 m with a 0.2% solution of Astro (permethrin [cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl) 2,2-dimethyl- (3-phenoxyphenyl) methyl ester]; FMC Corporation, Philadelphia, Pennsylvania, U.S.A.). The permethrin was first applied in August 2002 and reapplied in February and August 2003, prior to seasonal peaks in beetle flight (P. Svihra, unpublished data).

Monitoring of beetle responses

After the second permethrin application in February 2003 (day 204), a sticky trap of 30·30 cm steel hardware cloth coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan, U.S.A.) was affixed to each of the inoculated and mock-inoculated trees that had been sprayed with permethrin. Traps were hung adjacent to the inoculation and wounding site on each tree. The traps were placed only on the permethrin treated trees to subject the unsprayed trees to the full impact of any beetle attacks. Bark and ambrosia beetles were collected at days 275 (April), 300 (May) and 364 (July) and, subsequently, after traps were replaced after the August permethrin application, on days 450 (October) and 464 (November). Beetles from the last two dates were pooled due to the low catch in November. Beetles removed from traps were rinsed in mineral spirits, stored in 70% ethanol and then counted, tabulated by species, and sexed where possible. Beetle identification was based on a collection previously identified by Dr Donald E. Bright, using the appropriate key (Bright & Stark, 1973).

Evaluation, sampling and statistical analysis

All trees were monitored for signs and symptoms of *P. ramorum* infection beginning 100 days after inoculation and, subsequently, at the intervals described above. Disease stage categories were rated in order of increasing disease severity (or symptom development): asymptomatic, bleeding

only, bleeding plus beetle colonization, bleeding plus beetle colonization plus *H. thouarsianum* sporocarps, and dead (McPherson *et al.*, 2000; McPherson *et al.*, 2005). For each bleeding tree, the maximum vertical and horizontal distances from the point of inoculation were recorded on day 255, in early April. Canker size (m²) was calculated for this date using the average radius of apparent canker expansion, based on external symptoms.

Trees stems were inspected to approximately 2.5 m for boring dust and entrance holes in the bark and under the bark plug at each inoculation hole and examined for the presence of *H. thouarsianum* sporocarps. If entrance holes were numerous, they were estimated to the nearest multiple of ten, up to the observed maximum of 50 per tree.

We tested site as a factor in all analyses and, where it was found to be significant, the results are reported separately for each site. Otherwise, data from both field sites were pooled for all analyses. The effectiveness of permethrin in affecting the number of beetle attacks was evaluated using a repeated measures analysis of variance (ANOVA) to compare the cumulative numbers of beetle attacks for the two classes of bleeding inoculated trees, permethrin-treated and untreated, at days 275 (April), 300 (May), 333 (June), 364 (July) and 500 (December).

The effect of *P. ramorum* infection status on trap catch was analyzed using repeated measures ANOVA. Variables were tested to satisfy assumptions of normality and homogeneity of variances (Zar, 1996) by graphical analysis of residuals (Neter *et al.*, 1983). Nonhomogeneous variables were log-transformed to provide distributions that satisfied these assumptions. Analyses were first conducted to compare the log-transformed total beetle catch for all permethrin-treated inoculated and mock-inoculated trees. This analysis was then repeated for only bleeding versus nonbleeding inoculated trees. The seasonal response of each beetle species to *P. ramorum*-infected trees was analyzed with *t*-tests. A repeated measures ANOVA was used to examine the seasonal consistency of beetle trap numbers by species at days 275 (April), 300 (May), 364 (July) and 450 (October). The earliest two sampling dates were in the period of most active beetle flights, day 364 was in a period of relatively low beetle flight activity, and day 450 was at the end of the seasonal flight period. For differences to be considered significant, the value of alpha was set at 0.05. For differences to be considered marginally significant, the value of alpha was set at 0.1. The category of marginal significance was selected due to the known highly variable nature of the data collected in these studies.

Sex ratios of three ambrosia beetle species, *M. scutellare*, *M. dentigerum*, *Xyleborinus saxeseni* (Ratzeburg) and the bark beetle, *P. pubipennis*, were determined for each sampling date and were analyzed for differences from unity using *t*-tests in the JMP matched pairs platform (SAS, 2004).

To test for the influence of canker size on beetle catch, canker area measured at day 255 (early April) was regressed against log-transformed trap catch separately for each sampling date and for the year. To test for the effect of beetle tunneling on attraction to trees, the cumulative number of entrance holes (to that date) on each infected tree was regressed against the trap catch for each sampling date. We used a regression model to evaluate the relative contributions of both

canker size and the log-transformed cumulative number of beetle attacks to trap catch on infected trees.

Results

Phytophthora ramorum infections resulting from inoculation of coast live oaks produced the typical sequence of signs and symptoms of sudden oak death disease. Beetles had attacked 50% of the bleeding trees by December 2003, 17 months after inoculation. Sporophores of *H. thouarsianum* first appeared on seven of these trees within approximately 6 months of the first beetle attacks. Droplets of exudate that appeared on the bark initially within 10 cm of the inoculation hole confirmed the establishment of *P. ramorum*. Bleeding had developed on 11% of trees within 100 days, 60% within 200 days, and 81% within 500 days after inoculation. The surface area defined by the bleeding cankers, measured at day 255 (April), was in the range 0.008–1.13 m², with a mean size of 0.19 ± 0.03 m². Canker area changed little thereafter. Stem diameters (DBH) of inoculated trees did not significantly differ between the two sites ($t_{36} = 1.25$, $P = 0.11$).

Effects of permethrin treatment on beetle attacks

Beetles were attracted early in disease development to the bleeding cankers of both sprayed and unsprayed trees. Three of the unsprayed, inoculated trees were attacked by day 100, before these trees exhibited bleeding. Most of the initial beetle attacks occurred approximately 250–300 days after inoculation (April/May). Permethrin treatment delayed these attacks by approximately one and a half months. By mid-April (day 260), there were 215 beetle attacks on the untreated bleeding trees, but none on trees sprayed with insecticide. By day 300 (late May), when many of the permethrin-treated trees had come under attack, the additional 93 attacks recorded on the unsprayed trees were twice those of the permethrin-treated trees. The incremental numbers of attacks on the two trees groups at later dates were almost equal. By day 500 (December), beetle flights had ceased for the season. Largely because of the early attacks on unsprayed bleeding trees, the cumulative mean number of beetle attacks was consistently greater on unsprayed trees than on permethrin-treated trees (repeated measures ANOVA: $F_{1,53} = 6.86$, $P = 0.016$) (Fig. 2).

Permethrin treatment did not affect the incidence of bleeding ($\chi^2 = 0.05$, d.f. = 1,78, $P > 0.82$) or mean canker size ($t_{54} = 0.85$, $P > 0.39$). Despite the delay in attacks attributed to permethrin treatment, beetles had attacked similar percentages of bleeding trees (42% of sprayed bleeding trees and 53% of the unsprayed bleeding trees) by day 464 (November) ($\chi^2 = 0.68$, d.f. = 1,54, $P > 0.1$). Beetles did not attack any of the mock-inoculated or nonbleeding inoculated trees.

Beetle trap catch by species

Traps on inoculated trees accounted for 95% of the 2770 beetles caught in 2003. For all four sampling dates, more beetles were trapped on inoculated than mock-inoculated trees (repeated measures ANOVA: $F_{1,50} = 13.57$, $P < 0.001$)

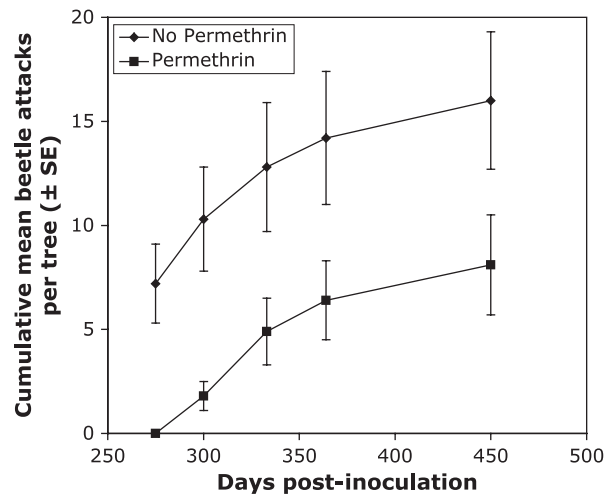


Figure 2 Cumulative number of beetle attacks per tree as a function of permethrin treatment for bleeding, inoculated coast live oak trees (mean ± SE). Permethrin was applied 220 and 385 days after inoculation. Evaluation days correspond to April, May, June, July and October.

(Fig. 3). All species but one were bark or ambrosia beetles in the family Scolytidae. The beetle species trapped (followed by percentages of the total catch) were: *M. scutellare* (29.3%), *X. saxeseni* (26.3%), *P. pubipennis* (25.5%), *M. dentigerum* (6.3%), *Xyleborus californicus* (Wood) (3.8%), *Gnathotrichus pilosus* (LeConte) (2.2%) and *Scobicia declivis* (LeConte) (Bostrichidae) (6.7%) (Table 1). By species, the total trap catch on inoculated trees was greater than on mock-inoculated trees for *M. scutellare* ($F_{1,53} = 5.836$, $P = 0.019$) and *X. californicus* ($F_{1,52} = 7.733$, $P = 0.0075$) and marginally greater for *G. pilosus* ($F_{1,52} = 3.64$, $P = 0.062$) and *X. saxeseni* ($F_{1,52} = 3.442$, $P = 0.069$).

There were significant differences between the two sites in total numbers of beetles trapped, with the mean catch at the Novato site being more than six-fold greater than that of the Nike site ($F_{1,53} = 6.26$, $P = 0.016$). The cumulative total of beetle attacks was higher throughout the year in the Novato than the Nike site ($F_{1,53} = 5.62$, $P = 0.021$). The greater abundance of beetles in the Novato site ranged from 2.3-fold greater than that of the Nike site for *X. californicus*, to 33- and 36-fold greater, for *X. saxeseni* and *P. pubipennis*, respectively. Despite these considerable differences in population sizes, the overall distribution of beetles and their responses to treatments did not differ in the two sites.

Sex ratios of two ambrosia beetle species varied seasonally. *Xyleborinus saxeseni* showed a female bias ($t_{1,47} = 2.6$, $P = 0.011$) that increased through the year, from 4:1 in May (day 300) to 12:1 by October (day 450). The sex ratio for *M. scutellare* varied greatly, with the first trap collection totals initially considerably male-biased (30:1), declining to 4:1 by day 364 (July) and increasing to 19:1 by day 450 (October). The magnitude of this variation precludes reporting an overall ratio. The sex ratio was male-biased in *M. dentigerum* (2:1; $t_{1,47} = 1.49$, $P = 0.07$) but did not differ from unity in *P. pubipennis* ($t_{1,48} = 1.1$, $P = 0.86$).

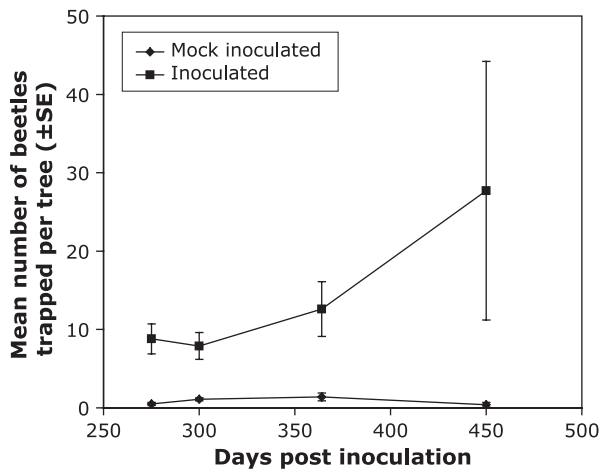


Figure 3 Total numbers of bark and ambrosia beetles caught on sticky traps as a function of inoculation status for the four collection dates in 2003 (mean \pm SE). Collection dates correspond to April, May, July and October, respectively.

Beetle landing rates

Trap catches showed differences for the periods before and after beetles first attacked the permethrin-treated trees. At the time of the first trap count in April (day 275), prior to any beetle attacks on the permethrin-treated trees, the inoculated trees had trapped more beetles than the mock-inoculated trees ($F_{1,53} = 15.78$, $P < 0.001$). The most abundant species, *M. scutellare*, was trapped in much greater numbers on inoculated than wounded trees during this first collection period ($F_{1,54} = 7.23$, $P = 0.009$). No other species showed significantly greater landing rates on inoculated trees at this time. The total beetle catch was also greater for all sampling dates on the inoculated trees that had developed bleeding cankers by day 255 (April) than for those that remained asymptomatic ($F_{1,34} = 9.27$, $P = 0.005$), which was primarily due to elevated numbers of *M. scutellare* ($F_{1,36} = 6.313$, $P = 0.0166$). This result confirmed that bleeding reliably identifies *P. ramorum* stem infections in *Q. agrifolia*.

The total trap catch increased as canker size increased ($F_{1,23} = 10.08$, $P = 0.004$) as it did for both *Monarthrum* species ($F_{1,26} = 13.18$, $P = 0.0012$ for *M. scutellare* and $F_{1,23} = 10.40$, $P = 0.0037$ for *M. dentigerum*). The trap catch

for all species at day 275 (April), which preceded the first beetle attacks on permethrin-treated trees, showed this response to trees with larger cankers ($F_{1,23} = 4.90$, $P = 0.037$). This relationship suggests that beetles exhibited a dose-dependent response to canker-derived kairomones.

Landing rates after beetle attacks

The cumulative number of beetle attacks on bleeding trees was also strongly associated with the total trap catch ($F_{1,23} = 50.48$, $P < 0.001$). When both canker size and the cumulative number of attacks were included as predictor variables of trap catch at each date, and parameter estimates were obtained for each variable, only the number of beetle attacks was a significant predictor variable for May (day 300) (overall $F_{2,23} = 8.55$, $P < 0.017$; attacks, $t_1 = 2.6$, $P = 0.016$, canker size, $t_1 = 1.03$, $P = 0.316$), July (day 364) (overall $F_{2,23} = 23.91$, $P < 0.0001$; attacks, $t_1 = 5.01$, $P < 0.0001$, canker size, $t_1 = 0.83$, $P = 0.415$), October (overall $F_{2,23} = 13.14$, $P = 0.0002$; attacks, $t_1 = 3.84$, $P = 0.0008$, cankers, $t_1 = 0.19$, $P = 0.85$) and for the year (overall $F_{2,23} = 24.78$, $P < 0.001$; attacks, $t_1 = 5.21$, $P < 0.001$, canker size, $t_1 = 0.34$, $P = 0.733$). Once beetles attacked the cankers on inoculated trees, their presence in the trees became the overriding influence on further attacks.

Discussion

Ambrosia and bark beetles selectively attacked *P. ramorum* cankers on half of the inoculated coast live oaks within 1 year after inoculation. The location of the attacks was restricted to the bark surface overlying the cankers. Canker expansion was defined by the distribution of exudate (bleeding) on the bark surface, although the earliest attacks occurred before any bleeding occurred. The only trees that died during the first year and a half of the study had been attacked by beetles relatively early, less than 10 months (300 days), after inoculation. Although inoculating trees probably led to accelerated disease progression, bark and ambrosia beetle colonization preceded the appearance of sporophores of secondary fungi, as is observed in natural infections (McPherson *et al.*, 2005).

Permethrin treatment reduced the total number of beetle attacks on *P. ramorum*-infected trees. At the time of the

Table 1 Total numbers of beetles caught in sticky traps in 2003, by species and tree treatment group

Month (days after inoculation)	April (275)		May (300)		July (364)		October (450)		Total	
	M	I	M	I	M	I	M	I	M (%)	I (%)
<i>Monarthrum scutellare</i>	8	299	16	147	11	248	5	118	40 (4.7)	812 (95.3)
<i>Xyleborinus saxeseni</i>	1	14	1	80	5	101	1	562	8 (1.0)	757 (99.0)
<i>Pseudopityophthorus pubipennis</i>	2	11	4	13	12	60	10	630	28 (3.8)	714 (96.2)
<i>Monarthrum dentigerum</i>	0	3	0	20	13	125	0	22	13 (7.1)	170 (92.9)
<i>Xyleborus californicus</i>	0	4	1	4	7	87	0	6	8 (7.3)	101 (92.7)
<i>Gnathotrichus pilosus</i>	0	4	2	18	4	31	1	4	7 (10.9)	57 (89.1)
<i>Scobicia declivis</i>	0	2	2	26	32	121	2	10	36 (18.5)	159 (81.5)
Totals	11	337	26	308	84	773	19	1352	140 (4.8)	2770 (95.2)

M, mock-inoculated (wounded control; $n = 19$ trees); I, inoculated ($n = 38$ trees).

study, information about preventing bark and ambrosia beetle attacks on hardwoods using permethrin was not available. Most likely because the permethrin application rate (0.2%) was lower than the maximum recommended rate (0.5%) for preventing bark beetle attacks on conifers, only partial protection was achieved. Despite the loss of efficacy of the permethrin, after 18 months the treated trees still had fewer beetle attacks than untreated trees. Because complete protection from attacks only lasted through the first 2 months of the beetle flight period, this result indicates that preventing beetle attacks early in the year significantly affected the cumulative impact of beetle attacks later throughout the year.

The failure of the permethrin treatment to prevent beetle attacks allowed us to test the hypothesis that attacks occurring soon after inoculation influenced subsequent beetle responses to these trees. During the period when the insecticide still protected cankers from beetle attacks, there was a positive relationship between canker surface area and trap catch. We ascribe this effect to production of volatile (kairomonal) attractants in the cankers, acting in a dose-dependent manner. As the insecticide lost effectiveness, beetles began to attack the cankers in greater numbers on trees that had previously trapped the most beetles. These were also the trees with larger cankers. Once beetles were tunneling into cankers, the number of entrance tunnels became the better predictor of further trap catches than canker surface area. These results are consistent with the hypothesis that beetles exhibited primary attraction (D. L. Wood, 1982) to compounds produced through the interaction of the pathogen with the host and that as they tunneled into the host material, secondary attraction to beetle-derived aggregation or sex pheromones provided an additional behavioral stimulus (D. L. Wood, 1982; Paine *et al.*, 1997). In addition to this behavioural evidence for host-produced attractant compounds, coast live oaks that were inoculated with *P. ramorum* have been shown to produce volatile compounds that were not detected in asymptomatic trees (Ockels, 2006). Because only half of the infected trees were attacked by beetles in the present study, it is likely that production of volatile kairomones varied between trees. Accelerated disease progression therefore may be dependent on the attacks of a limited number of pioneering beetles.

The male-biased sex ratio for both *M. scutellare* and *M. dentigerum*, especially early in the flight season for the former species, is consistent with reports that the males of these species are the primary colonizers that produce aggregation pheromones (Bright & Stark, 1973). Furthermore, these were the only species that were trapped in greater numbers on trees with larger cankers. Because *M. scutellare* was the most abundant species in traps, particularly early in the season, these beetles may initiate the colonization process for other beetle species and secondary saproxylic insects that follow, similar to the preconditioning effect proposed for ambrosia beetles colonizing trembling aspen *Populus tremuloides* (Hammond *et al.*, 2001).

The four most abundant ambrosia beetle species, *M. scutellare*, *M. dentigerum*, *X. californicus* and *X. saxeseni*, colonize oaks, tanoaks and some other hardwoods (Chamberlain, 1958; Furniss & Carolin, 1977; S. L. Wood, 1982). Both *Monarthrum*

species are native to western North America. *Xyleborinus saxeseni* was reported to be introduced from Europe (S. L. Wood, 1982) but may have a circumpolar distribution (Wood & Bright, 1992; Atkinson & Peck, 1994) and *X. californicus* probably has an Asian origin (S. L. Wood, 1982).

Three lines of evidence support our hypothesis that saproxylic beetle colonization of coast live oaks influences the course of the disease by accelerating disease progression and altering the structural integrity of *P. ramorum*-infected trees. First, preferential colonization of *P. ramorum*-infected coast live oaks by bark and ambrosia beetles suggests that infection of host tissues by the pathogen generates volatile compounds that beetles use as cues to initiate host colonization. During the earliest stages of beetle attacks, infected trees appear to be robust, with healthy foliage and none of the symptoms and signs of general decline expected for trees with a disease that has a high likelihood of causing mortality. During this phase, the only visible symptom of infection is stem bleeding. Attraction of bark and ambrosia beetles to volatiles released from *P. ramorum*-infected trees may explain the densely aggregated beetle attacks on *P. ramorum* bark cankers (Fig. 1), a behaviour that has not previously been reported for these temperate ambrosia beetles in natural forest settings. Even if initial attraction of ambrosia beetles to *P. ramorum*-infected trees is relatively weak, the production of aggregation pheromones by the first beetles to tunnel into the bark may induce a localized mass attack. Furthermore, when ambrosia beetles attack their more typical host material, such as recently killed trees, including those that died after *P. ramorum* infection, the entrance holes along the stem occur at a lower density than when these beetles attack *P. ramorum*-caused cankers (B. A. McPherson, unpublished observation). This pattern may be regulated by production of anti-aggregation pheromones, as suggested for the striped ambrosia beetle *Trypodendron lineatum* (Nijholt, 1970).

Second, approximately half of all the *P. ramorum*-infected coast live oaks in Marin County, California, forest study sites had been colonized by ambrosia and bark beetles at any time and almost every bleeding tree that died had been previously colonized when the foliage was still green and functional (McPherson *et al.*, 2005). Median modelled survival declined considerably for infected trees that had been colonized by ambrosia and bark beetles. The primacy of beetle attacks in the process was indicated by the finding that trees with both beetle attacks and *H. thouarsianum* sporophores had median estimated survival times that did not differ from trees with beetle attacks alone.

Finally, in a separate but related study, the role of bark and ambrosia beetle infestations in the introduction and/or stimulation of wood inhabiting filamentous fungi from *P. ramorum*-infected coast live oak trees in Marin Co., California, is under investigation (N. Erbilgin, P. Bonello, B. A. McPherson and D. L. Wood, unpublished data). Fungal species that were isolated from live beetle-colonized trees, but not from beetle-free infected trees, include *Geosmithia* sp., which are associated with bark beetles in Europe (Kolarik *et al.*, 2005) and *Trametes versicolor*, a broadly distributed hardwood decay fungus. This evidence suggests that preferential colonization of *P. ramorum*-infected coast live oaks by

bark and ambrosia species accelerates tree mortality by introducing fungi deep into the sapwood, directly (by vectoring) and/or indirectly (by creating tunnels).

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