# A study of landing behaviour by the walnut twig beetle, *Pityophthorus juglandis*, among host and nonhost hardwood trees in a northern California riparian forest

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**Abstract**
1 The host selection behaviour of the walnut twig beetle, *Pityophthorus juglandis*, was assessed by monitoring the landing rates of the beetles with sticky sheet traps on the host and nonhost hardwood branches.

2 Sticky sheet traps were deployed for 8 weeks from 6 June to 2 August. 2017 in the Putah Creek Riparian Reserve, Davis, *CA*. Branches from host northern California black walnut, *Juglans hindsii*, were paired with branches from six nonhost hardwood species.

3 The landing rate of *P. juglandis* (412 beetles trapped/8 weeks; 389 on host branches, 23 on nonhost branches) was significantly greater on the host branches for all nonhost hardwoods except *Populus fremontii*. Proportional comparisons of beetle presence also revealed a significant preference for the host branches compared with all but two nonhost species, *Acer negundo* and *P. fremontii*.

4 Capturing *P. juglandis* without the use of an aggregation pheromone was a rare event, underscoring the difficulty of studying the initial phases of host selection behaviour in bark beetles. Unbaited funnel traps adjacent to selected host trees in the experiment only captured five individuals over a 19-week period. None were captured in traps adjacent to nonhost trees.

5 This study provided evidence that *P. juglandis* discriminates between host and nonhost branches while in-flight. This directed flight behaviour is likely informed by the recognition of both host and nonhost volatile cues.

6 This study established an ecological context for the development of a semiochemical-based repellent system for protecting walnut trees from future attacks from this invasive bark beetle.

**Keywords** Bark beetle, coleoptera, *Geosmithia morbida*, host selection behaviour, *Juglans hindsii*, northern California black walnut, *Pityophthorus juglandis*, Scolytidae, thousand cankers disease, walnut pest.

## Introduction

Detecting and responding to chemicals is a crucial means of interacting with the environment for many species of insects (Kaissling, 1971). This is especially true for the bark beetles (Coleoptera: Scolytidae, *sensu* Bright, 2014), a group of insects that rely extensively on the interpretation of volatile signals (*e.g.*, aggregation pheromones) and cues (*e.g.*, compounds in the

volatile profile of host or nonhost trees or pheromone components of other insect species) to direct their host searching and selection behaviour (Wood, 1980, 1982; Silverstein, 1981; Raffa *et al.*, 1993; Borden, 1997; Raffa, 2001; Seybold *et al.*, 2018). Probably the most understood aspect of bark beetle chemical ecology is the production of and behavioural responses to aggregation pheromones (Wood, 1982). However, bark beetles may recognize and respond to a complex suite of compounds beyond just the aggregation pheromone signal. For instance, Byers *et al.* (1984) reported on the inhibition of western pine

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beetle, *Dendroctonus brevicomis* LeConte, by the pheromones from two sympatric species of *Ips*, *I. pini* (Say) and *I. paraconfusus* Lanier. The authors posited that the recognition of heterospecific pheromonal components reduces interspecific competition. Similarly, production of antiaggregation pheromones, like verbenone in the mountain pine beetle, *D. ponderosae* Hopkins, and the southern pine beetle, *D. frontalis* Zimmermann (Rudinsky *et al.*, 1974), are also thought to mediate intraspecific competition over the limited phloem resource in a given host tree (Byers *et al.*, 1984). Species of bark beetles also recognize volatile cues (kairomones) from host trees (Wood, 1982; Hobson *et al.*, 1993); however, there is mounting evidence of bark beetle recognition of nonhost cues as well (Zhang & Schlyter, 2004). These nonhost volatiles, generally extracted from angiosperm foliage and bark, have been inhibitory when presented to coniferophagous scolytids (Wilson *et al.*, 1996; Byers *et al.*, 1998; Huber *et al.*, 2001; Jactel *et al.*, 2001; Fettig *et al.*, 2009). Thus, bark beetles rely on numerous compounds, both signals and cues, simultaneously to direct host colonization behaviour (Graves *et al.*, 2008).

Bark beetles follow a four-step behavioural process for host colonization: (i) *dispersal phase* in which beetles emerge from the brood tree and fly to a new host, beetles respond to host stimuli and/or pheromones; (ii) *selection phase* in which beetles respond to host cues to accept or reject the potential host, prior to and/or after landing, acceptance is defined by sustained feeding in the host; (iii) *concentration phase* in which aggregation pheromone is produced by actively colonizing beetles, drawing in conspecifics; and (iv) *establishment phase* in which both bark beetles and fungal inoculum have colonized sufficiently to ensure adequate host tissue death (in many cases whole tree death) for successful brood development (Wood, 1982). Bark beetles are most vulnerable during the dispersal, and selection phases as these represent periods when adult beetles are exposed before entering the subcortical habitat, and as a result, significant mortality has been reported for bark beetles during these behavioural phases (Stark & Dahlsten, 1970; Byers, 1995; Raffa, 2001). Understanding a particular bark beetle pest's chemical ecology can provide insights on how to effectively manage its populations and protect trees with semiochemical repellents (Silverstein, 1981; Borden, 1997; Seybold *et al.*, 2018).

Two prevailing hypotheses explain the search behaviour of the initial colonizers or pioneers (*i.e.*, the first few beetles to a potential host tree). The first argues that scolytids employ undirected flight and landing behaviour (a.k.a., random landing) in which beetles are believed to respond primarily to visual cues that direct flight towards a tree (Graves *et al.*, 2008). Identification of host or nonhost and subsequently the selection phase then occurs *via* short-range olfaction and/or gustation. The second hypothesis argues that beetles respond actively, in-flight, to long-range olfactory cues for directed flight and landing behaviour. Beetles may exhibit some level of host recognition prior to landing with the ultimate selection occurring post-landing (Graves *et al.*, 2008). Directed flight search behaviour may be dominated by semiochemical disruption whereby olfactory cues from nonhost trees may lead to avoidance until host cues are detected. Evidence for the earliest stages and events of host colonization has been difficult to obtain, especially for hardwood-attacking species.

The walnut twig beetle, *Pityophthorus juglandis* Blackman (Bright, 1985; Seybold *et al.*, 2019), is a good model system for studying the initial stages of host colonization by a hardwood bark beetle. *Pityophthorus juglandis* attacks walnut (*Juglans*) and wingnut (*Pterocarya*) trees and vectors a phloem-pathogenic fungus, *Geosmithia morbida*, in the thousand cankers disease (TCD) insect-pathogen complex (Tisserat *et al.*, 2009; Hishinuma *et al.*, 2016; Seybold *et al.*, 2016). Although native to North America, *P. juglandis* and its fungal associate have recently expanded well beyond their historic range in Arizona, New Mexico, and northern Mexico (Cranshaw, 2011; Zerillo *et al.*, 2014; Rugman-Jones *et al.*, 2015; Seybold *et al.*, 2016). The current distribution of *P. juglandis* includes Chihuahua, Mexico, nine U.S. western states (Arizona, California, Colorado, New Mexico, Idaho, Nevada, Oregon, Utah, and Washington) (Bright Jr., 1981; Wood & Bright Jr., 1992; Cranshaw, 2011; Tisserat *et al.*, 2011; Seybold *et al.*, 2012, 2013b, 2016), and seven U.S. eastern states (Tennessee, Pennsylvania, Virginia, North Carolina Ohio, Maryland, and Indiana) (Grant *et al.*, 2011; Seybold *et al.*, 2012, 2013b, 2016; Fisher *et al.*, 2013; Hadziabdic *et al.*, 2014; Marshall, 2015). The beetle and pathogen have also established populations in Italy (Faccoli *et al.*, 2016; Montecchio *et al.*, 2016; Moricca *et al.*, 2018).

*Pityophthorus juglandis* colonizes and develops in all species of *Juglans*, including North American black walnuts and agriculturally important English walnut, *J. regia* L. (Flint *et al.*, 2010; Seybold *et al.*, 2016; Hishinuma, 2017; Hefty *et al.*, 2018). Together with *P. juglandis* and *G. morbida* cause progressive top-down crown dieback, often culminating in the mortality of walnut trees. Infestations of *P. juglandis* threaten urban shade trees, timber production, agricultural nut production, and native walnuts in riparian stands that are important for wildlife and riparian forest ecology (Seybold *et al.*, 2019). These threats are particularly concerning in California. It is crucial to find effective management strategies to mitigate the potential impacts of *P. juglandis* and TCD.

The objective of this study was to provide an ecological context for semiochemical disruption of *P. juglandis* host colonization. We specifically tested the hypothesis landing rates of *P. juglandis* on nonhost hardwood species, even when in close proximity to host trees, would be reduced compared with landing rates on the hosts. This would provide evidence that *P. juglandis* actively discriminates between host and nonhost species prior to landing. This directed flight and landing behaviour is potentially mediated by the recognition of components of both host and nonhost volatile profiles. The chemical components of these profiles could be exploited to further enhance attraction to the pheromone lure or as inhibitory repellents to protect walnut trees from the impacts of *P. juglandis* and TCD.

## Methods

### Study site

This study was conducted at the University of California Davis Putah Creek Riparian Reserve in Davis, California (N 38.523543°, W 121.785156° to N 38.516850°, W 121.758155°). The Putah Creek Reserve is a riparian-forested area along the northern bank of Putah Creek, which flows from

**Table 1** Common, binomial, and family names, plus the size ranges of tree species included in an experiment to assess the landing rates of walnut twig beetle, *Pityophthorus juglandis*, in a riparian forest habitat in northern California (Putah Creek Riparian Reserve, Davis, California)

| Common name | Binomial name | Family | *n* | Range of DBH[a] (cm) |
|---|---|---|---|---|
| Northern California black walnut | *Juglans hindsii* Jeps. Ex R.E. Sm. | Fagaceae | 60 | 4.5–55.7 |
| River red gum | *Eucalyptus camaldulensis* Denh. | Myrtaceae | 10 | 8.9–26.4 |
| Boxelder maple | *Acer negundo* L. | Sapindaceae | 10 | 4.8–21.3 |
| Fremont cottonwood | *Populus fremontii S. Watson* | Salicaceae | 9 | 15.2–97.6 |
| Oregon ash | *Fraxinus latifolia* Benth. | Oleaceae | 10 | 9.1–20.5 |
| Valley oak | *Quercus lobata* Neé | Fagaceae | 10 | 8.1–57.1 |
| Red willow | *Salix* spp.[b] | Salicaceae | 10 | 11.9–48.4 |

[a]DBH = diameter at breast height, measured roughly 1.3 m above the ground.
[b]Likely *S. laevigata* and *S. gooddingii*, and possibly *S. lasiolepis*.
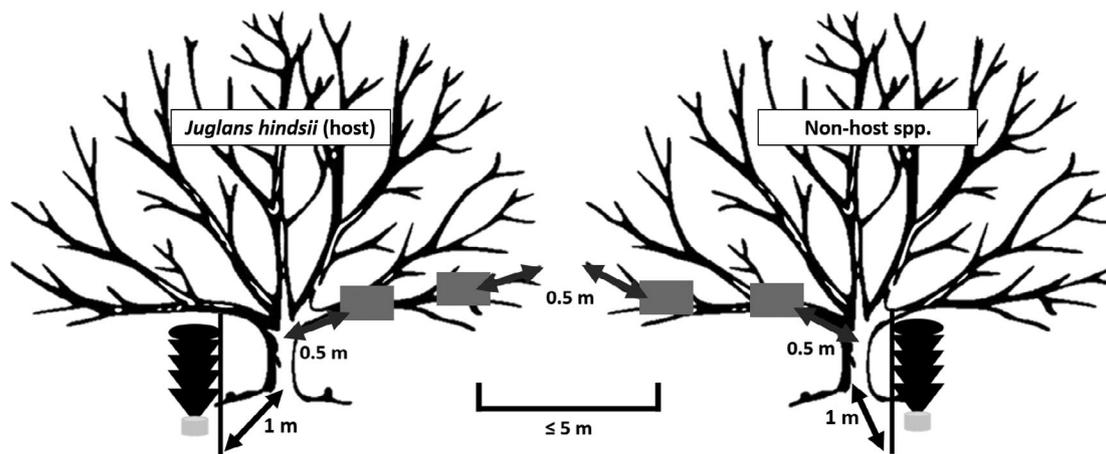


**Figure 1** Diagram of host and nonhost branch pairings for a study of the landing behaviour of the walnut twig beetle, *Pityophthorus juglandis*. Sticky sheet traps (grey rectangles), were placed approximately 0.5 m from the terminus (distal) and 0.5 m from the stem (proximal) of the selected branches. Selected branches of host and nonhost trees were within five metres of one another. Unbaited, four-unit Lindgren funnel traps were placed within one-metre of the stem of a host and nonhost trees. One pair of funnel traps was deployed for one pair of each of the six nonhost hardwood species included in the study. Sticky sheet traps were deployed from 6 June to 2 August 2017, and funnel traps were monitored weekly from 15 June to 26 October 2017, Putah Creek Riparian Reserve, Davis, California.

west to east out of the Vaca Mountains (Inner Coast Range) out of Lake Berryessa Reservoir (Solano and Yolo Counties). Although much of Putah Creek between Winters and Davis, California has been altered anthropogenically beginning in the late 1800s (Matheny *et al.*, 1991), the reserve currently supports a fairly representative riparian forest, consistent with what existed pre-European settlement in the Central Valley (Griffin & Critchfield, 1976; Katibah, 1984). The study site segment of Putah Creek has a significant number of trees of northern California black walnut, *Juglans hindsii*, along with six other hardwood species, including red river gum, *Eucalyptus camaldulensis*, boxelder maple, *Acer negundo*, Fremont's cottonwood, *Populus fremontii*, Oregon ash, *Fraxinus latifolia*, valley oak, *Quercus lobata*, and red willow, *Salix laevigata* (Table 1; Hort Science, 1997). The previous sampling from the reserve confirmed active infestations of *P. juglandis* and *G. morbida* (Oren *et al.*, 2018). These data were corroborated by a visual survey of the reserve, which revealed significant crown dieback, especially among the larger diameter *J. hindsii* trees.

The early stages of host selection behaviour of *P. juglandis* were assessed by comparing the landing rates on host *J. hindsii* branches paired with branches of nonhost hardwood species.

Ten hosts/nonhost pairs were established for each of six nonhost species (only nine pairs for *P. fremontii*) for a total of 59 branch pairings (*n* = 118 branches surveyed). Nonhost species (Table 1) included in the study were *E. camaldulensis*, *A. negundo*, *P. fremontii*, *F. latifolia*, *Q. lobata*, and at least two species of willow were used, likely *S. laevigata* and *S. gooddingii*, however, *S. lasiolepis* is also reported to occur in the reserve in low numbers (HortScience, 1997). Branches selected for the host/nonhost pairs were all between 2.5 and 10 cm in diameter, approximately 1–4 m off the forest floor, and within 5 m of one another (Fig. 1). For the host trees, only branches that did not have signs of entrance/emergence holes, staining, and were on trees with no visible signs of crown dieback from TCD were selected to minimize the potential influence of aggregation pheromone from already attacked branches on *P. juglandis* landing rate.

*Trap placement and insect handling*

Following branch selection, two sticky sheet traps (21.6 cm × 27.9 cm acetate sheets, Apollo Transparency Film,

product# VPP100C, ACCO Brands Inc., Lincolnshire, Illinois, covered in Stickem Special™, Seabright Laboratories, Emeryville, California) were wrapped around the branch, one approximately 0.5 m from the branch terminus (distal end) and the second approximately 0.5 m from the main stem (proximal end) (*n* = 236 traps, Fig. 1). Sheets were pinned onto two small corks fastened to each branch with wood glue. Traps were deployed for 8 weeks from 6 June to 2 August 2017. At the end of the experiment, prior to removal of the traps, the branch aspects (top, right, bottom, and left) were labelled on the acetate sheets (relative to the axis projecting from the main stem to the periphery). The traps were then transported back to the lab where all *P. juglandis* and associated insects were removed by using forceps and droplets of xylene (Avantor Performance Materials, Inc., Product #8668–16, lot #K51B05, Centre Valley, Pennsylvania) to dissolve some of the trap adhesives on the specimens. Species identification was confirmed, and the sexes of the beetles were separated by using a Zeiss Stemi 2000 Stereomicroscope at 40× (Fisher Scientific, Atlanta, GA, USA). Once recorded (number of specimens/aspect/trap), the insects were placed into vials with xylene to further dissolve the adhesive and for storage. Associated insects targeted in this study included subcortical beetles and potentially predaceous Neuroptera (*i.e.*, Chrysopidae and Hemerobiidae). These were identified at least to family and to species when possible.

As a secondary measure of *P. juglandis* flight activity near the host and nonhost trees, a pair of four-unit Lindgren funnel traps were placed adjacent to the stems of host and nonhost trees (Seybold *et al.*, 2013a). One pair of funnel traps was deployed for each of the six hosts/nonhost species pairings. The funnel traps were attached to the top of 2.44-m-tall metal conduit poles. Each pole was placed over a one-metre long piece of steel rebar hammered into the ground one-metre from the stem of each tree (Fig. 1). No aggregation pheromone lures or any other semiochemicals were attached to the funnel traps. Funnel traps were emptied weekly (15 June to 26 October 2017) beyond the duration of the study by using standard methods (Seybold *et al.*, 2013a). As with the sticky sheet traps, all specimens of *P. juglandis* and associated insects were identified and recorded.

*Species identifications*

Most specimens of Coleoptera were identified by JPA and SJS. A few scolytid specimens were sent to Dr. Donald E. Bright, Colorado State University, Fort Collins, Colorado for identification and/or confirmation. All specimens of Neuroptera were identified to family by JPA. For species-level identification of Chrysopidae and Hemerobiidae, the authors consulted Catherine A. Tauber, University of California Davis, Davis, California, who examined a subset of our material. We separated and tallied the sexes of several species of Coleoptera, including *P. juglandis* (Seybold *et al.*, 2013a), *Xyleborinus saxeseni* (Ratzeburg) (all females; males are flightless), and *Nathrius brevipennis* (Mulsant) (Linsley, 1963). Trees utilized in the study were identified by JPA and SJS except for *E. camaldulensis* and *S. laevigata*, which were identified to species by Dr. Ellen Dean, University of California Davis Centre for Plant Diversity, Davis, California.

*Data handling and statistical analyses*

All analyses were conducted using R statistical software R (v. 3.4.4) within the RStudio platform (R Core Team, 2019). The presence or absence of *P. juglandis* was compared on host *vs.* nonhost trees. The presence was defined as $\geq 1$ *P. juglandis* for each sticky sheet trap. A trap where *P. juglandis* was present was assigned a 1, whereas a trap without *P. juglandis* was assigned a 0. The proportion of sheets with *P. juglandis* was compared for all pooled hosts and nonhosts and individually for each of the six hosts/nonhost comparisons. Proportional comparisons were made by using the [prop. Test()] function in R (R Core Team, 2019).

*Pityophthorus juglandis* landing rates were analysed by using zero-inflated negative binomial models with the [zeroinfl()] function with a 'negbin' distribution and a 'logit' link in the pscl package (V 1.5.2, Jackman, 2017). Initially, landing rates were modelled by using both a negative binomial generalized linear model (GLM) and the zero-inflated model. Factors in the models included tree species (two levels: host and nonhost), trap placement (two levels: proximal and distal), sheet aspect (four levels: top, right, bottom, and left), and *P. juglandis* sex (two levels: male and female). Models were compared by using the Akaike information criterion (AIC) values and Vuong's likelihood ratio tests (Zuur *et al.*, 2009; Yang *et al.*, 2017). The simplest adequate model was selected in each case. Landing rates were compared initially for all host and nonhost trees pooled, and then subsequently for each of the individual six nonhost species pairs. *Post-hoc* least-squares means comparisons were conducted for significant factors in the final models with the [emmeans()] function in the emmeans package (V 1.4.2, Lenth *et al.*, 2019).

Too few *P. juglandis* were captured in the funnel traps to warrant any statistical analysis, therefore, only the raw catch data are presented here. Finally, simple means comparisons ($\chi^2$ tests) were conducted to compare the mean number of selected insect species that landed on *J. hindsii vs.* the nonhost hardwoods. All statistical tests were considered significant for *P*-values $\leq .05$ (*i.e.*, $\alpha = 0.05$).

## Results

A total of 412 *P. juglandis* (198 males and 214 females) were captured on the sticky sheet traps during this study. Of the 412, 389 were recovered from traps on the host branches, and 23 were recovered from traps on the nonhost branches. The proportion of traps positive for *P. juglandis* on the host trees was significantly greater than on the nonhost traps overall and in all cases of individual comparisons except for *J. hindsii vs. A. negundo* ($\chi^2_1 = 0.133$, $P = 0.358$) and for *J. hindsii vs. P. fremontii* ($\chi^2_1 = 0.709$, $P = 0.199$) (Table 2).

In analysing the landing rates, the zero-inflated negative binomial (ZINB) model fits the data better than the negative binomial (NB) model in every case (Table 3). In most cases, the optimized ZINB model produced both a lower AIC value and was statistically significant when compared by using the Vuong's likelihood ratio test. Tree species (host *vs.* nonhost) and trap placement (proximal *vs.* distal) were frequently included and statistically significant in the final models. Trap aspects left, and the top was significant in the *P. fremontii* model (Table 3), but no other

**Table 2** The proportion of sticky sheet traps that were positive for walnut twig beetle, *Pityophthorus juglandis*, when placed on branches of host *versus* nonhost trees hardwood trees

| Branch pairing | *n* | Host | Non-host | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|
| All *Juglans hindsii* vs all nonhosts | 236 | 50/118 | 12/118 | 29.948 | **<0.001** |
| *J. hindsii* vs *Eucalyptus camaldulensis* | 40 | 16/20 | 2/20 | 17.071 | **<0.001** |
| *J. hindsii* vs *Acer negundo* | 40 | 6/20 | 4/20 | 0.133 | 0.358 |
| *J. hindsii* vs *Populus fremontii* | 36 | 5/18 | 2/18 | 0.709 | 0.199 |
| *J. hindsii* vs *Fraxinus latifolia* | 40 | 5/20 | 0/20 | 3.657 | **0.028** |
| *J. hindsii* vs *Quercus lobata* | 40 | 11/20 | 2/20 | 7.293 | **0.003** |
| *J. hindsii* vs *Salix* spp. | 40 | 9/20 | 2/20 | 4.514 | **0.017** |

Traps were placed on trees in the Putah Creek Riparian Reserve, in Davis, California. (6 June – 2 August 2017). Proportion comparisons for host *versus* nonhosts were made for each branch pairing by using the prop. Test() function in the stats package in the R Statistical software. All bold *P*-values indicate statistical significance ($\alpha = 0.05$).

aspects were significant. Sex of *P. juglandis* was not a significant factor in any model.

Pairwise, least-squares means (estimated marginal means) comparisons indicated highly significant (*P*-values <.001) differences in the mean numbers of *P. juglandis* landing on traps on *J. hindsii* trees *vs*. all nonhost trees for pooled host *vs*. pooled nonhost sticky traps ($z$ ratio$_6$ = 6.901, $P < 0.001$). Highly significant differences in the mean number of beetles caught were also found for the following host/nonhost pairings: *J. hindsii vs. E. camaldulensis* ($z$ ratio$_{14}$ = 4.808, $P < 0.001$); *J. hindsii vs. A. negundo* ($z$ ratio$_5$ = 3.848, $P < 0.001$); *J. hindsii vs. Q. lobata* ($z$ ratio$_7$ = 4.129, $P < 0.001$); and *J. hindsii vs. Salix* spp. ($z$ ratio$_{10}$ = 3.382, $P = 0.001$; Fig. 2). The mean number of *P. juglandis* that landed on host branches was also significantly greater than the number that landed on *F. latifolia* as well ($z$ ratio$_9$ = 1.957, $P = 0.05$), although not highly significant. The only pairwise comparison that was not significantly different was the comparison between *J. hindsii* and *P. fremontii* ($z$ ratio$_{13}$ = 0.003, $P = 0.997$; Fig. 2).

The number of *P. juglandis* recovered from proximal and distal traps was not statistically significant for sticky sheet traps placed on the host or nonhost trees. However, the trap placement factor was still retained in most of the final models (Table 3) as the AIC values were lower when the factor was included. The model factor sex of *P. juglandis* (male *vs*. female) was only significant in the *F. latifolia* model, where a slightly greater number of males were caught than females ($z$ ratio$_9$ = 1.957, $P = 0.05$). Trap aspect was not significant in any of the models, and no differences in the mean number of beetles among trap aspects were observed.

Despite monitoring funnel traps from 15 June to 26 October 2017 (19 week), only five adult *P. juglandis* were caught (3 males, 2 females). All beetles were recovered from traps placed adjacent to *J. hindsii* trees. Trap catch breakdown was as follows: one female from the *J. hindsii – F. latifolia* pair; 1 male from the *J. hindsii – Q. lobata* pair; 1 female from the *J. hindsii – E. camaldulensis* pair; and 2 males from the *J. hindsii – Salix* spp. pair.

Total sticky trap catches for the associated insect species of interest were summed for all host trees and all nonhost trees (Table 4). Means comparisons were only conducted for those associated species where at least 100 individuals were recovered. These species included *Xyleborinus saxeseni* (Coleoptera: Scolytidae), *Nathrius brevipennis* (Coleoptera: Cerambycidae),

*Petalium californicum*, *Ptilinus* sp., *Priobium punctatum* (all Coleoptera: Anobiidae), *Chrysoperla carnea* species complex (Neuroptera: Chrysopidae), *and Hemerobius sp.* (Neuroptera: Hemerobiidae) (Table 4). The catches of these seven species were averaged by the tree for all hosts ($n = 59$) and nonhost trees ($n = 59$). A greater number of *X. saxeseni* were caught on the pooled *J. hindsii* branches ($3.53 \pm 0.62$) compared with the pooled nonhost branches ($1.32 \pm 0.18$) ($\chi^2_1 = 18.252$, $P < 0.001$). The mean comparison tests also revealed a preference for *J. hindsii* branches by both of the Neuroptera. More specimens from the *Chrysoperla carnea* species complex landed on host tree branches ($5.83 \pm 0.33$) *vs*. nonhost tree branches ($2.52 \pm 0.18$) ($\chi^2_1 = 15.716$, $P < 0.001$), and more *Hemerobius* sp. landed on host branches ($2.80 \pm 0.18$) *vs*. nonhost branches ($0.80 \pm 0.07$) ($\chi^2_1 = 25.667$, $P < 0.001$). No differences in the means were found for the four other species analysed (all wood borers in the Anobiidae and Cerambycidae).

## Discussion

The results of this study provide evidence in favour of the hypothesis that *P. juglandis* discriminate between host and nonhost tree branches to some degree prior to landing. Thus, we reject the hypothesis of random landing search behaviour for this system (Graves *et al.*, 2008). Although attractive host kairomonal cues might have elicited an elevated landing rate by *P. juglandis* on *J. hindsii* branches, the significantly lower landing rates on traps on the nonhost branches may also suggest that repellent nonhost volatiles may have suppressed the landing rate on those traps (Fig. 2). The latter was true for host comparisons with all nonhosts pooled and for five of the six nonhosts individually. A future comparative test between landing on nonhost branches *vs*. non-emitting surrogate branches (Lona *et al.*, 2020) would be interesting to pursue to further establish the role of nonhost repellency in this system.

Our results corroborate a study in a *Juglans* germplasm collection by Hishinuma (2017), who reported landing rate preferences by *P. juglandis* for branches of several species of *Juglans*, indicating that the beetles responded prior to landing to cues (likely chemical) present among multiple walnut tree species. The results of our study are also consistent with those of Homicz *et al.* (*in review*), who reported significant differences in the number of *P. juglandis* recovered from identical traps

**Table 3** Final zero-inflated negative binomial models selected following model optimizations and comparisons for the landing rate of the walnut twig beetle, *Pityophthorus juglandis*

| Nonhost species | Formula | df | AIC | Count model factors | z ratio | P-value | Zero-inflated model factors | z ratio | P-value |
|---|---|---|---|---|---|---|---|---|---|
| All nonhost vs *Juglans hindsii* | TotalBeetles ~ species + trap Placment \| trap placement | 6 | 1735.03 | Species | −12.007 | **<0.001** | Trap placement | −0.154 | 0.877 |
| *Quercus lobata* | TotalBeetles ~ species * trap placement \| species | 7 | 260.57 | Trap placement<br>Species<br>Trap placement | −1.87<br>−1.464<br>2.221 | 0.62<br>0.143<br>**0.026** | Species | −0.053 | 0.958 |
| *Fraxinus latifolia* | TotalBeetles ~ species + trap Placment + trap aspect + sex \| 1 | 9 | 45.11 | All factors | 0.0–1.0 | All >0.05 | \|1 | Na | Na |
| *Acer negundo* | TotalBeetles ~ species \| trap placement | 5 | 439.16 | Species<br>Trap placement | −5.856<br>2.194 | **<0.001**<br>**0.028** | Trap placement | −2.484 | **0.013** |
| *Eucalyptus camaldulensis* | TotalBeetles ~ species + tree * trap placement + tree * trap aspect + tree * sex \| 1 | 14 | 419.4 | All other factors (+ all interactions) | −1.166 to 0.678 | All >0.05 | \|1 | Na | Na |
| *Populus fremontii* | TotalBeetles ~ species + trap placement + trap aspect \| species + trap placement + trap aspect | 13 | 81.08 | Species<br>Trap placement<br>Trap aspect left<br>Trap aspect right<br>Trap aspect top | −0.443<br>0.084<br>−2.082<br>0.064<br>−2.466 | 0.658<br>0.933<br>**0.037**<br>0.949<br>**0.014** | Species<br>Trap placement<br>Trap aspect left<br>Trap aspect right<br>Trap aspect top | 0.081<br>0.102<br>−0.102<br>0.081<br>−0.049 | 0.935<br>0.919<br>0.919<br>0.935<br>0.961 |
| *Salix* spp. | TotalBeetles ~ tree + trap placement + trap aspect + sex \| tree | 10 | 388.72 | Species<br>Trap placement<br>All other factors | −2.094<br>−2.31<br>−1.688 to 0.932 | **0.036**<br>**0.021**<br>All >0.05 | Species | 2.044 | **0.041** |

The formula for each selected model is given for each nonhost species pairing. Model factors included: Species (*Juglans hindsii* [host], nonhost); Trap Placement (proximal, distal); Trap Aspect (top, right, bottom, left); and Sex (male, female). These models were used to compare the mean landing rates of *Pityophthorus juglandis* on the host and nonhost hardwood trees in the Putah Creek Riparian Reserve in Davis, CA. (6 June to 2 August 2017). All *P*-values in bold represent significant factors in the model ($\alpha = 0.05$). AIC = Akaike information criterion. An estimator of the relative quality of a statistical model. These values were used as a part of the model optimization and selection process (Zuur *et al.*, 2009)
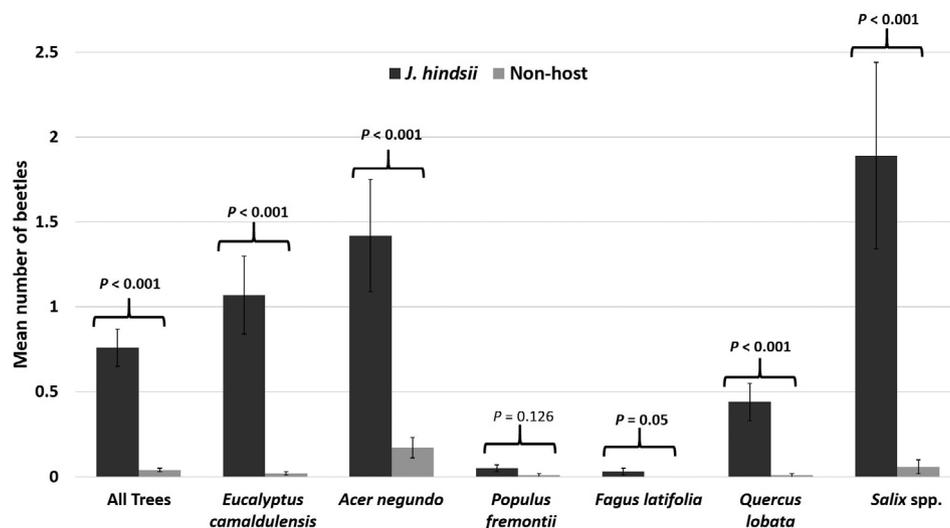
**Figure 2** Pairwise comparisons of the estimated marginal mean (least-squares means) numbers of *Pityophthorus juglandis* that landed on sticky sheet traps placed around branches of paired *Juglans hindsii* (host) and six nonhost hardwood species, 6 June to 2 August 2017, Putah Creek Riparian Reserve, Davis, California. Brackets above-paired histogram bars denote the *P*-value for each pairwise test ($\alpha = 0.05$).

**Table 4** Number of associated insect species of interest captured in a study of the landing behaviour of the walnut twig beetle, *Pityophthorus juglandis*

| Order | Family | Species | *Juglans hindsii* | Nonhost | Total |
|---|---|---|---|---|---|
| Coleoptera | Scolytidae | *Xyleborinus saxeseni* | 208 | 78 | 286 |
| | Scolytidae | *Trypophloeus thatcheri* | 3 | 1 | 4 |
| | Scolytidae | *Hypothenemus eruditus* | 3 | 2 | 5 |
| | Scolytidae | *Scolytus rugulosus* | 0 | 2 | 2 |
| | Scolytidae | *Dendrocranulus cucurbitae* | 3 | 12 | 15 |
| | Scolytidae | *Hylocurus* sp. | 6 | 5 | 11 |
| | Scolytidae | *Pseudohylesinus* sp. | 0 | 2 | 2 |
| | Scolytidae | *Hylesinus* prb. *Oregonus* | 0 | 4 | 4 |
| | Curculionidae | *Stenomimus* sp. | 4 | 2 | 6 |
| | Curculionidae | *Rhyncolus* sp. | 5 | 7 | 12 |
| | Cerambycidae | *Xylotrechus insignis* | 0 | 2 | 2 |
| | Cerambycidae | *Nathrius brevipennis* | 76 | 98 | 174 |
| | | *N. brevipennis* sex ratios M:F[a] | 57:12 | 56:10 | 143:22 |
| | Cerambycidae | *Phymatodes vulneratus* | 1 | 1 | 2 |
| | Anobiidae | *Petalium californicum* | 481 | 636 | 1117 |
| | Anobiidae | *Ptilinus* sp. | 114 | 122 | 236 |
| | Anobiidae | *Priobium punctatum* | 45 | 56 | 101 |
| | Laemophloeidae | *Narthecius simulator* | 2 | 0 | 2 |
| | Laemophloeidae | Unknown species | 17 | 19 | 36 |
| | Buprestidae | Unknown species | 3 | 0 | 3 |
| | Bostrichidae | Unknown species | 1 | 3 | 4 |
| Neuroptera | Chrysopidae | *Chrysoperla carnea* species complex | 344 | 149 | 493 |
| | Hemerobiidae | *Hemerobius* sp. | 165 | 47 | 212 |
| Raphidioptera | Raphidiidae | Unknown species | 0 | 4 | 4 |

Data were recovered from sticky sheet traps placed around branches of trees located in the Putah Creek Riparian Reserve, Davis, California (6 June to 2 August 2017). Trap captures were pooled for branches of all *Juglans hindsii* (host) and for six other hardwoods (nonhost).
[a]We were unable to separate the sexes of 2 of the specimens of *N. brevipennis* because of damage from the trap adhesive.

on branches of hosts *vs*. nonhosts in four of their six pairings. In the other two instances, no differences in landing rate were found for *J. hindsii vs. Q. lobata* or for *J. hindsii vs. A. negundo*. In the latter study, a release device for the aggregation pheromone (3-methyl-2-buten-1-ol) was placed beneath the traps on branches of both the host and nonhosts (the same six nonhost hardwood species pairings were made, also in the Putah

Creek Riparian Reserve). Thus, some level of host/nonhost discrimination was maintained by *P. juglandis*, even in the context of the artificial lure ('secondary' attraction).

Neither *P. juglandis*, nor any of the other subcortical beetle species showed a preference for either the proximal or distal trap placement. For *P. juglandis*, we expected to see more beetles landing on the proximal traps as these traps were placed around

larger diameter sections of the branch, and despite the common name, *P. juglandis* is typically found on larger diameter branches and the main stem (Seybold *et al.*, 2016). The lack of trap placement preference indicates the beetles did not discriminate among the branch diameter ranges presented in this study. Similarly, the associated insects did not discriminate by branch diameter.

The suppression of *P. juglandis* landing rate by nonhost volatile emissions (Fig. 2) suggests the potential for isolating and identifying the behavioural chemical cues responsible for the effect. Tree species of primary interest include the non-native *E. camaldulensis* and two native North American species, *P. fremontii* and *Salix* spp. (Fig. 2 and Homicz *et al.*, *in review*). Eucalyptus is a genus well known for strong, distinctive terpenoid aromas (Yang *et al.*, 2004), and it is possible that *P. juglandis* is repelled or interrupted by one or more of these highly volatile compounds. It would be interesting to explore the mechanism (*e.g.*, active recognition of the cue by a dedicated receptor or antagonism of a receptor tuned to another stimulus) driving this observed behavioural response, especially given the obvious lack of evolutionary history between *P. juglandis* (North America) and species of eucalyptus (Oceania).

*Populus fremontii* and various species of *Salix* co-occur with the putative ancestral host of *P. juglandis*, Arizona walnut, *J. major* (Torr.) (Rugman-Jones *et al.*, 2015; USGS, 2017). All three species of *Salix*, (*S. laevigata*, *S. gooddingii*, and *S. lasiolepis*) reported from Putah Creek Riparian Reserve (HortScience, 1997), are partially sympatric with *J. major* (USGS, 2017). Thus, *P. juglandis* may have had the opportunity to evolve to recognize cues from *P. fremontii* and/or one or more *Salix* spp. as it sought to discriminate among riparian host trees. Once identified, these semiochemical cues may be useful as an integrated pest management (IPM) tool for protecting walnut trees from colonization by *P. juglandis*.

Interestingly, we did not find significant differences in landing rates of *P. juglandis* related to sex or trap aspect (top, left, bottom, or right). Given that male *P. juglandis* initiate colonization and produce the aggregation pheromone (Seybold *et al.*, 2016), we expected to find more males than females in our trap catches, which we anticipated would reflect the early steps of host selection behaviour. We only found this to be the case in one instance, the *F. latifolia* pairing, and given the low number of beetles caught, this result may have been skewed. However, the balanced sex ratios of sticky sheet and funnel trap catch of *P. juglandis*, particularly in the sticky sheet trap catches, supports the interpretation that the landing responses were to host or nonhost cues and not to aggregation pheromones produced in the underlying branches or in adjacent portions of the host trees. The male-produced aggregation pheromone tends to result in a female-biased ratio of trap captures (Chen & Seybold, 2014). In no instance did we find a beetle preference for trap aspect on the branches. We had expected to see a pattern given the findings from Hishinuma (2017) who reported a preference for the underside (bottom) of the branches. In two instances of our study, the host branch was found to have been mass attacked by the end of the experiment (as evidenced by bark staining and entrance holes, Seybold *et al.*, 2013a, 2016). These attacks may have inflated *P. juglandis* landing rates on the host traps given the natural production of aggregation pheromone accompanying

such attacks. Both instances occurred in *J. hindsii* and *A. negundo* pairings. These results were not removed from the analysis; however, as an elevated landing rate of *P. juglandis* was also recorded on the corresponding nonhost traps. This may have indicated a level of 'spill-over' effect from the host/nonhost branches because they were particularly close together, and likely had some balancing effect within the analysis. Despite our best efforts, it is unlikely that our experimental design was able to completely mute the effect of natural pheromone on the landscape. Overall, both sticky sheet and funnel trapping confirmed that capturing *P. juglandis* during the early stages of host colonization is a rare event, and both sexes appear to be active in searching for and landing on all spatial aspects of host branches.

Twenty-four associated insect species of interest were recorded from the sticky sheet traps in this study (Table 4). *Petalium californicum* (Anobiidae) was the most numerous of all the associated insects (1117 individuals). We reported eight other species of bark beetles or ambrosia besides *P. juglandis*, including the invasive ambrosia beetle *Xyleborinus saxeseni*, and the invasive bark beetle *Hypothenemus eruditus* (only females of both species were captured; males are flightless). We have trapped large numbers of both of these subcortical beetles in commercial walnut (*J. regia*) orchards in California (Seybold *et al.*, 2016; Lona *et al.*, 2020), but have had very few observations of their activity in association with native riparian black walnut. The large catch of *X. saxeseni* landing on live host branches is somewhat confusing as this species is known to prefer declining branches/trees (Seybold *et al.*, 2016). However, 44% (91/208) of the total catch came from one of the two mass attacked *J. hindsii* branches, which may have begun to decline and ferment during the 8 week study period. This may explain both the high number of *X. saxeseni* caught and the observed preference for *J. hindsii* over the *P. juglandis* nonhost hardwoods, which can likely serve as ambrosia fungal hosts for the 'polyphagous' *X. saxeseni* (Seybold *et al.*, 2016). The three species of Anobiidae caught in this study, another group of beetles that prefer dead and/or dying trees, did not show a preference for *J. hindsii* or the pooled nonhost hardwoods (Table 4).

The invasive cerambycid *Nathrius brevipennis* was encountered in relative abundance during the study (174 specimens, Table 4). This species has been observed landing on several species of walnut in large numbers (Seybold *et al.*, 2016; Hishinuma, 2017); however, it has typically been observed in commercial *J. regia* walnut orchards (Lona *et al.*, 2020). A close association of *N. brevipennis* with adventive *J. regia* in the USA is logical considering the Eurasian origin of both the beetle (Linsley, 1963) and the tree (Aradhya *et al.*, 2006). Indeed, Linsley (1963) suggests that *N. brevipennis* may have been introduced to California in 'Persian' walnut (*i.e.*, *J. regia*). To our knowledge, this is the first report of *N. brevipennis* from a native forest ecosystem in California, although Linsley (1963) notes southern California black walnut, *J. californica*, among its hosts. Despite the documented association of *N. brevipennis* with *Juglans*, we did not observe a landing rate distinction between *J. hindsii* and the pooled nonhost hardwood species. Linsley (1963) suggests that the beetle is polyphagous on many hardwoods and even conifers. The male-skewed sex

ratio (approx. 5:1; Table 4) during our landing assays is consistent with similar sex ratios reported by Lona *et al.* (2020) on their landing traps from two orchard locations in California. Another interesting result was the apparent preference of both specimens from the *Chrysoperla carnea* species complex and *Hemerobius* sp. for *J. hindsii vs.* the other hardwood species. We suspect this observation may be in response to volatiles related to tree stress, likely induced by *P. juglandis* attacks, and are sceptical that the response is directly related to the beetle's presence.

As *P. juglandis* and TCD continue to damage and kill walnut trees across the landscape in the invaded range in North America, especially in California, it is important that effective management strategies be developed. This study provides evidence that the host selection behaviour of *P. juglandis* is actively directed by cues from the host and nonhost volatile profiles, thus providing an ecological context for exploring semiochemical interruption/repellency in this system. Using semiochemicals to disrupt and even repel bark beetles as a tool for protecting trees is an increasingly used approach for natural resource managers, particularly in coniferophagous bark beetle systems (Seybold *et al.*, 2018). Based on the observed directed landing host selection behaviour employed by *P. juglandis*, this system is a prime candidate for implementing a semiochemical based management strategy for a hardwood-attacking bark beetle.

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## Data availability statement

Data available upon request from the authors.

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