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# Host selection behavior mediated by differential landing rates of the walnut twig beetle, *Pityophthorus juglandis*, and associated subcortical insect species, on two western North American walnut species, *Juglans californica* and *J. major*

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## Abstract

The walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), vectors a phytopathogenic fungus, *Geosmithia morbida* Kolařík et al. (Hypocreales), which causes thousand cankers disease (TCD) in walnut (*Juglans* sp.) and wingnut (*Pterocarya* sp., both Juglandaceae) trees. We investigated an early point in disease inception in two walnut species – *Juglans californica* S. Wats. and *Juglans major* (Torr. ex Sitsgr.) Heller – native to riparian forests of the western USA by comparing *P. juglandis* flight and landing responses to small-diameter branch sections. Twenty unbaited branch sections (10 each of *J. californica* and *J. major*) were presented in a completely randomized design to populations of *P. juglandis* at the USDA Agricultural Research Service, National Clonal Germplasm Repository (NCGR) *Juglans* collection located at Wolfskill Experimental Orchards (Winters, CA, USA) and at the California State University, Chico, Agricultural Teaching and Research Center (ATRC, Chico, CA). These assays were carried out within a 4- to 6-year period when weekly flight surveys with aggregation pheromone-baited multiple funnel traps revealed that *P. juglandis* flight activity–abundance was higher at the NCGR than at the ATRC. For the landing rate assays, adhesive-coated acetate sheets were wrapped around the branch sections and exchanged weekly. Three assays were completed at the NCGR (assays 1–3), whereas one assay was completed at the ATRC (assay 4). Landing rates on these traps were compared between *J. californica* and *J. major*. Two additional assays (5 and 6) were completed at the NCGR to compare responses to branch sections of *J. californica* and to similarly sized cardboard tubes (negative control). All six assays were completed over a 4-year span during the 4- to 6-year weekly flight survey period. Pooled landing rates of male and female *P. juglandis* (assays 1–4) demonstrated a preference by both sexes for *J. californica* over *J. major*. In assay 5 there was no preference by males or females for *J. californica* over the negative control, perhaps due to the low flight activity–abundance of *P. juglandis* during the assay. When repeated at a time of higher flight activity–abundance (assay 6), male and female landing rates on *J. californica* exceeded those on the negative control. Females of the invasive fruit-tree pinhole borer

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†Steven J. Seybold passed away on November 15, 2019. We dedicate this work to him.

(an ambrosia beetle), *Xyleborinus saxeseni* (Ratzeburg), and an invasive bark beetle, *Hypothenemus eruditus* Westwood (both Coleoptera: Scolytidae), showed relatively higher flight responses than either sex of *P. juglandis* during most assays, suggesting higher population densities of these two other invasive species at the two orchards or a greater sensitivity to host volatiles. *Xyleborinus saxeseni* and *H. eruditus* preferred to land on *J. major* over *J. californica* and on *J. californica* over the negative control. Similarly, an invasive longhorned beetle, *Nathrius brevipennis* (Mulsant) (Coleoptera: Cerambycidae), showed a significant preference for *J. major* over *J. californica*, but not for *J. californica* over the negative control. More male *N. brevipennis* were trapped than females at both study sites [sex ratio ranged from 5:1 (assay 6) to 39:1 (assay 4)], and flight occurred only in the spring and early summer months. Another ambrosia beetle trapped at the NCGR and ATRC, *Xyleborus affinis* Eichhoff, represented the first records of this species from western North America. In summary, flight responses recorded in some of our assays for *P. juglandis* and several other subcortical insects on *Juglans* indicate that host preference by these insects may be determined by long-range olfactory cues that do not involve pheromones.

## Introduction

The walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), vectors a phytopathogenic fungus, *Geosmithia morbida* Kolařík et al. (Hypocreales) (Kolařík et al., 2011), which causes thousand cankers disease (TCD) in walnut (*Juglans* sp.) and wingnut (*Pterocarya* sp., both Juglandaceae) trees (Seybold et al., 2016). Walnut trees are used commercially in North America for the production of nuts and high-quality lumber (Leslie et al., 2010), and ornamentally in urban areas or along rural roads (Graves et al., 2009). Walnut also provides a food and cover source for various mammals and birds (Horton, 1949; Quinn, 1990; Rink, 1990; Williams, 1990).

Through feeding, construction of brood galleries, or both behavioral activities, *P. juglandis* transfers the spores of the fungus to the phloem of host trees (Seybold et al., 2013a). Affected trees have dark, wet stain spots on the bark surface and cankers in the phloem around *P. juglandis* entrance holes (Graves et al., 2009). As the numbers of beetle entrances accumulate, numerous cankers in the branches and stem can result in crown dieback and tree mortality (Tisserat et al., 2009; Seybold et al., 2013a, 2019).

*Pityophthorus juglandis* (Bright, 1981) and *G. morbida* (Kolařík et al., 2011; Tisserat et al., 2011) were first collected and described from populations in the western USA, and since 2010, they have also spread to the eastern USA (Seybold et al., 2013a, 2016). Surveys in the western USA suggest that *P. juglandis* may be the only subcortical insect to carry *G. morbida* (Kolařík et al., 2017). There have been reports from the eastern USA that other subcortical insects may vector *G. morbida* in walnut, but the phoresy rates have been low, transmission has not been demonstrated experimentally, and the insects were mostly species that target moribund trees (Juzwik et al., 2015). TCD has also spread to Italy where it occurs in five regions (Faccoli et al., 2016; Moricca et al., 2018).

Native walnut species in the western USA include southern California black walnut, *Juglans californica* S. Wats., northern California black walnut, *Juglans hindsii* (Jeps.) Rehder, Arizona black walnut, *Juglans major* (Torr. ex Sitsgr.) Heller, and little walnut, *Juglans microcarpa* Berland. Native to the eastern USA is eastern black walnut, *Juglans nigra* L., which is grown widely for wood production (Williams, 1990), and butternut, *Juglans cinerea* L., which is a valuable component and mast producer in the mixed hardwood forest (Rink, 1990). English walnut, *Juglans regia* L., is native to central Asia and is used commercially worldwide for its nut production. Phylogeographic analyses suggest that *J. major* is the most likely native host for *P. juglandis* (Rugman-Jones et al., 2015) and *G. morbida* (Zerillo et al., 2014). Both pest organisms may have co-evolved with *J. major* throughout its native range (Zerillo et al., 2014; Rugman-Jones et al., 2015) leading to relatively low susceptibility of *J. major* to the disease elements (Table 1). Although TCD in California was first recognized in 2008 (Flint et al., 2010), collection records of *P. juglandis* beginning in 1959 suggest that the disease may have been active in the state for much longer. The impact of the disease on *J. californica* planted in a USDA germplasm repository in northern California has been substantial, whereas the impact on *J. regia* in this repository and in California's commercial orchards has been relatively modest (Table 1; Hishinuma, 2017; Seybold et al., 2019).

The impact of TCD has been evaluated by subdividing the disease into the susceptibility of host material to fungal infection and to the reproductive success of the beetle (Table 1). *Juglans nigra*, *J. hindsii*, and *J. californica* are highly susceptible to *G. morbida* based on lesion length or area on inoculated branches and stems, whereas *J. major* is

**Table 1** Synthesis of measures of host susceptibility to walnut twig beetle, *Pityophthorus juglandis*, and *Geosmithia morbida* from the literature. Impact of thousand cankers disease among host trees has been assessed by evaluating the relative rates of landing and reproductive success of *P. juglandis* among the hosts, as well as the development of *G. morbida* cankers among species of juvenile host plants or excised branches

| | *Pityophthorus juglandis* | | | | | | *Geosmithia morbida* |
|---|---|---|---|---|---|---|---|
| | Landing rate | | | Reproductive success (no. *P. juglandis* progeny) | | Tree mortality | Development[1] |
| Host | Hishinuma (2017) – chapter 2 | Hishinuma (2017) – chapter 3 | Hishinuma (2017) – chapter 4 | Hefty et al. (2016) | Hefty et al. (2018) | Hishinuma (2017) – chapter 1 | Utley et al. (2013) |
| *Juglans ailantifolia* | – | – | Intermediate | – | Low | – | Low |
| *J. californica* | – | Highest | Highest | – | Highest | Highest | High |
| *J. cathayensis* | – | – | – | – | High | – | – |
| *J. cinerea* | – | – | Lowest | No difference | Low | – | Intermediate |
| *J. hindsii* | No difference | Low | High | – | High | Intermediate | High |
| *J. major* | – | Low | Intermediate | – | Low | Lowest | Lowest |
| *J. mandshurica* | – | – | – | – | Low | – | Low |
| *J. microcarpa* | – | Lowest | Intermediate | – | Lowest | – | High |
| *J. nigra* | – | Lowest | – | No difference | Intermediate | Moderate | Highest |
| *J. regia* | No difference | – | Low | – | Low | Low | Intermediate |
| *Pterocarya* sp. | – | – | Lowest | – | Lowest | – | – |
| *Carya illinoensis* | – | – | – | – | Unsuccessful | – | Lowest |
| *C. ovata* | – | – | – | – | Unsuccessful | – | Lowest |

[1]Number and development of *G. morbida* cankers in laboratory/greenhouse assays.

less susceptible (Table 1; Utley et al., 2013). Similarly, reproductive success (number of progeny) of *P. juglandis* is greater in *J. nigra*, *J. hindsii*, and *J. californica* than in *J. major* (Table 1; Hefty et al., 2016, 2018). Host selection by *P. juglandis* is a critical set of behavioral steps when spores of *G. morbida* are introduced to the phloem of walnut or wingnut trees, which results in TCD. However, little is known about the role that host selection by *P. juglandis* plays in TCD impact (Hishinuma, 2017).

Host plant selection by bark beetles is influenced by factors such as visual, olfactory, tactile, and gustatory cues (Raffa et al., 2016). The process may begin with undirected or directed flight and landing based on long-range olfactory signals (Graves et al., 2008). These signals may include behavioral chemicals from host and non-host plants, as well as from conspecific or heterospecific bark beetles (Graves et al., 2008). Directed flight may be followed by shorter-range walking behavior guided by olfaction or feeding guided by gustatory stimulation and deterrence (Graves et al., 2008). After a host plant is selected, *P. juglandis* males produce an aggregation pheromone, which attracts both sexes (Seybold et al., 2015), resulting in increased initiation of brood galleries by new males.

In this study, we sought to determine whether *P. juglandis* uses olfactory cues in its initial detection of the host, thereby validating the importance of host plant selection by *P. juglandis* in the vectoring of *G. morbida*. Volatile compounds released from live branches on trees of different species of *Juglans* may elicit varying landing rates by *P. juglandis* (Hishinuma, 2017). Hishinuma (2017) also studied flight responses by *P. juglandis* to cut branch sections from various species of *Juglans* by baiting the branch sections with aggregation pheromone lures, and then comparing the *P. juglandis* landing rates. These studies showed a higher landing preference for *J. californica* than for *J. major*. However, landing responses on branch sections have not been investigated in the absence of pheromone lures, which might have influenced the observed preferences. We investigated differential landing rates of *P. juglandis* and various associated insects on unbaited branch sections of *J. californica* and *J. major*, two key walnut hosts of *P. juglandis*. By following this approach, we sought to establish the rationale for pursuing a future comparative chemical analysis of bark volatiles from the two hosts in hopes of discovering chemical attractants or repellents.

## Materials and methods

### Study sites

Experiments were conducted in two walnut orchards: (1) a multi-species planting at the USDA Agricultural Research Service, National Clonal Germplasm Repository

(NCGR) *Juglans* collection at Wolfskill Experimental Orchards in Winters, CA (Solano County) (38°30′00.4″N, 121°58′37.1″W), and (2) a commercial-style English walnut, *J. regia*, orchard at the California State University, Agricultural Teaching and Research Center (ATRC) in Chico, CA (Butte County) (39°41′41.3″N, 121°49′13.1″W). The NCGR site contained approximately 1 000 *Juglans* trees representing 15 species planted in 25 rows of 40 trees each, whereas the ATRC site contained approximately 2 200 trees of one species planted in 44 rows of approximately 50 trees. Forty of the rows were planted with the cultivar Chandler grafted on Paradox (*J. regia* × *J. hindsii* or *J. californica*) rootstock; four rows were planted with multiple cultivars, which included 12 named cultivars (e.g., Durham, Forde, Gillet, Hartley, Ivanhoe, Payne, etc.), as well as numerous unnamed experimental crosses from the UC Davis (UCD) Walnut Breeding Program. Rows were aligned from west to east at both sites.

#### Seasonal flight activity of walnut twig beetle

In order to determine *P. juglandis* flight activity within each orchard, four-unit funnel traps with wet cups (Lindgren, 1983; Seybold et al., 2013b) were maintained at each study site. At the NCGR, two traps were placed on one pole on the southern edge of the orchard, and two were placed on one pole near the center of the orchard. At the ATRC, four traps (one per pole) were placed on the eastern (two traps: one among cv. Chandler and one among multiple cultivars) and western (two traps: one among Chandler, one among multiple cultivars) edges of the orchard and two (one per pole) were placed in the interior of the multiple cultivar block. These funnel traps were suspended on ca. 3-m-long stainless-steel conduit poles and baited with the male-produced aggregation pheromone of *P. juglandis* (Seybold et al., 2013b; Chen & Seybold, 2014). Traps were attached to the pole at 1.5 and 2.75 m (NCGR) and at 2.75 m only (ATRC). The different arrangement of traps on the poles was a consequence of the earlier establishment of the NCGR survey site as part of a broader orchard survey for *P. juglandis* in California. Approximately 100 ml of ethanol-free marine antifreeze (Winter Safe Antifreeze, product no. 31200SLVV3; Kinpak, Montgomery, AL, USA) containing propylene glycol were added into the trap cups to immobilize any captured *P. juglandis*. Once per week, the contents of the cups were collected by pouring antifreeze laden with insects through conical paper paint strainers with nylon mesh (Basecoat/Clearcoat Strainers, Fine 190 Micron 1000CT, product no. Gerson-10601; Toolrage, Dillsburg, PA, USA) and placing the filtered contents in plastic bags (Seybold et al., 2013b). Collected samples were frozen and then sorted in the laboratory to determine the total number of male and female *P. juglandis* caught each week, as well as associated insects such as the fruit-tree pinhole borer, *Xyleborinus saxeseni* (Ratzeburg), the minute bark beetle, *Hypothenemus eruditus* Westwood (both Coleoptera: Scolytidae), and *Nathrius brevipennis* (Mulsant) (Coleoptera: Cerambycidae).

#### Collection and handling of host material for landing rate assays

Methods for the landing rate assays generally followed the procedures described by Hishinuma (2017) with some modifications as described below. Six flight landing assays were conducted during the spring and fall of 2015 and 2016, and spring of 2019 (assay 1: 5 May to 6 June 2015, NCGR; assay 2: 29 August to 24 October 2015, NCGR; assay 3: 10 April to 24 June 2016, NCGR; assay 4: 13 April to 21 June 2016, ATRC; assay 5: 27 August to 29 October 2016, NCGR; and assay 6: 8 April to 21 June 2019, NCGR). In the first four assays, *P. juglandis* landing rate was measured on translucent adhesive-coated acetate sheets (see below for details) surrounding branch sections of *J. californica* and *J. major* (Figure 1). In the two final assays (fall 2016 and spring 2019), *P. juglandis* landing rate was measured on the same type of sheets surrounding branch sections of *J. californica* and a negative control, which was a 1.5 × 18 inch (i.e., 3.8 × 45 cm) cardboard mailing tube (product no. 37005-OD; LePage's 2000, Taylor, MI, USA) cut to a length of 42 cm (Figure 2).

For assays 1–5, branches of *J. californica* and *J. major* ranging in diameter from 1.3 to 4.7 cm were cut from trees at the UCD Hutchison Road *Juglans* Collection, New Stuke Block, HQ *Californica*, calif 97-040, Davis, CA (Yolo Co.) (38°32′21.2″N, 121°47′45.1″W) and at the NCGR (Table S1). Tree accessions in the NCGR from which we collected branches were generally not clones, but different individuals collected from various field sites in Arizona and California. Assays 1 and 2 relied on branch sections cut from one tree of each species, whereas assays 3–4 (*J. major*) and assays 3–5 (*J. californica*) involved branch sections cut from 10 trees of each species (Table S1), most of which had different geographic origins in their respective ranges. Branches on the orchard trees were selected for cutting without regard to location in the tree crown, but based on their diameter and the absence or low density of *P. juglandis* entrance or emergence holes. The high population density of *P. juglandis* and the high susceptibility of *J. californica* to colonization in these areas made it difficult to locate branches of *J. californica* that did not show evidence of these holes (Hishinuma, 2017). These branches were cut into approximately 42 cm sections (Figure 1) and placed in a walk-in freezer (−25 °C) for at least 3 days to kill any live insects in the branches. All branches were retrieved from the freezer and placed overnight in a

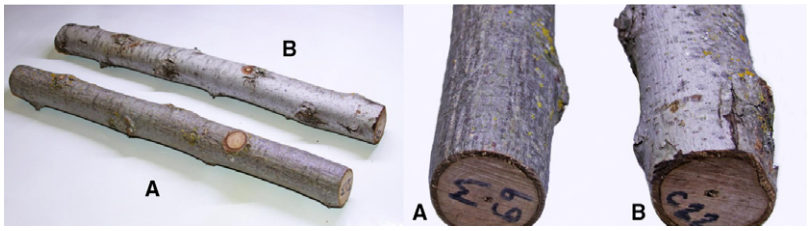


**Figure 1** Branch sections of two *Pityophthorus juglandis* host plants used in the flight landing assays 1–4: (A) *Juglans major* (3.8 cm mean diameter, 42.1 cm mean length, n = 40) and (B) *J. californica* (3.7 cm mean diameter, 42.2 cm mean length, n = 40). Photographs by SM Hishinuma (UCD Department of Entomology and Nematology). [Colour figure can be viewed at wileyonlinelibrary.com].

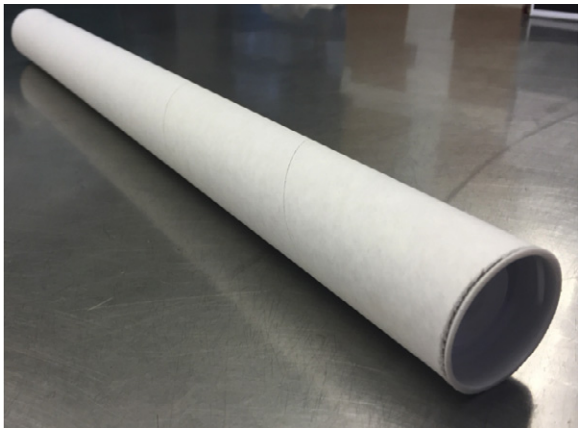

**Figure 2** Cardboard tube (4 cm diameter, 42 cm long) used as a negative control to compare *Pityophthorus juglandis* landing rate on a host, *Juglans californica* (3.8 cm mean diameter, 40.7 cm mean length, n = 10) vs. a non-host without volatiles. Photograph by ID Lona. [Colour figure can be viewed at wileyonlinelibrary.com].

walk-in cold room (4.4 °C) to thaw prior to their placement in the field. For assay 6, branches of the same dimensions were cut from 12 wild trees across the native range of *J. californica* (Table S2). These were also cut into sections (41.3–43.7 cm long, 2.7–5.3 cm diameter) and frozen as described above.

#### Preparation of host material and placement in the field

For each assay, thawed branch sections of *J. californica* and *J. major* were examined again in the laboratory for any pre-existing entrance holes or old galleries of *P. juglandis*. Branch sections showing a high density of *P. juglandis* entrance or emergence holes were discarded. Ten branch sections each of *J. californica* and *J. major* with zero or a low density of these holes were selected for assays 1–5. Depending on the *P. juglandis* activity on the available branches, the low densities ranged from 0 to 50 holes, and the holes were marked with a red paint pen. However, if we were unable to find sufficient branch sections with low densities for a given assay, a small number of branch sections that exceeded 50 *P. juglandis* holes were used (14 of 50 sections of *J. californica* in five assays, and 0 of 40 sections of *J. major* in four assays). However, these were checked carefully to ensure that no beetles were present, by removing a small amount of bark around the hole and galleries with a razor blade. Branch sections from two of the wild *J. californica* trees (assay 6, entries 7 and 8 in Table S2) were discarded when evidence of extremely high prior infestation by *P. juglandis* was discovered in the laboratory. There was evidence of limited prior infestation on the branch sections ultimately selected for assay 6 (15 of 20 branch sections had 5 or fewer old entrance/emergence holes; the remaining branch sections had 6, 25, 27, 30, and 91 of these holes).

All branch sections of both species were sealed at each end with wax (product no. 203-060-005; Gulf, Roswell, GA, USA) to reduce desiccation. Eyehole screws (no. 6 zinc-plated screw eyes; CrownBolt, Aliso Viejo, CA, USA) were attached to each end of the branch sections, which were then placed into mesh screen bags (100 × 100 mesh, 0.01 mm [T-316 stainless steel 100x100-0-0045-T-316-PW] Screen Technology Group, Santa Fe Springs, CA, USA), and the bags were sealed with hot glue. The bags were used to prevent incidental colonization by responding beetles.

For each of assays 1–5, 20 branch sections or cardboard tubes (10 for each treatment) were placed in the field. Branch sections or tubes were assigned randomly to 1 of 20 trapping stations (completely randomized design) located primarily between NCGR accessions of northern California black walnut, *J. hindsii*, in the northeastern corner of the NCGR orchard, and between Chandler *J. regia*, in the center of the ATRC orchard. Each screened branch section was suspended horizontally with wires from a stainless steel conduit pole so that it hung ca. 1.5 m above the orchard floor (Figure 3). Cork stoppers were glued to the outside of each mesh bag with wood glue. Adhesive-coated, translucent acetate sheets (27.9 × 43.2 cm, Apollo Transparency Film, product no. VPP100C; ACCO Brands,

**Figure 3** *Juglans* branch section surrounded by a mesh screen bag hung from a 1.5-m-tall stainless steel conduit pole in the field (left). An adhesive-coated acetate sheet was placed around the bagged branch section (right) to trap adult *Pityophthorus juglandis* and associated insects as they attempted to land. Photograph by ID Lona. [Colour figure can be viewed at wileyonlinelibrary.com].

Lincolnshire, IL, USA) were placed around each branch section and held in place with push pins attached to the cork stoppers (Figure 3). The use of the cork stoppers minimized any further damage to the bark of the branch sections. The trap adhesive used on the acetate sheets was Stikem Special (regular formulation; Seabright Laboratories, Emeryville, CA, USA). Henceforth we will refer to these caged branch section preparations wrapped with stikem-coated acetate sheets as 'branch section traps' and they will be differentiated by host type.

Every 7 days (in most instances), branch sections were re-randomized among the trapping stations. Prior to re-randomization, all insects of interest were removed from the adhesive-coated acetate sheets in the field and, during periods of peak flight, the acetate sheets were removed from the field for laboratory assessment of any remaining insects of interest and replaced with fresh adhesive-coated acetate sheets. We conducted the first assay for four 7-day cycles, and the others for 8–11 7-day cycles. Assay 6 was modified by establishing an initial round of 10 *J. californica* branch sections (five 7-day cycles) followed by a second round of six 7-day cycles where the experiment was expanded to include 10 more branch sections from the same trees. The latter branch sections had remained in freezer storage during the first five cycles. Thus, the second round of the experiment included 30 elements re-randomized among 30 trap stations: 10 each of (1) the original branch sections ('old'), (2) the second set of branches ('new'), and (3) the cardboard tubes. During the first round, the 20 elements were also re-randomized among the 30 trap stations. Branch sections were removed at the end of the assays and placed into cold storage so that they could be evaluated in the laboratory (see below).

#### Handling of insects and acetate sheets in the laboratory

Adhesive-coated acetate sheets were examined under a heat lamp or under a dissecting microscope (40× magnification), and insects of interest were excised or lifted from acetate sheets and transferred to vials of xylene to dissolve the trap adhesive. After soaking for at least 20 min, insects were separated from the pieces of acetate sheet and dried on paper towels or wipes and washed twice in 100% ethanol. Insects were then transferred into vials of 100% ethanol for storage. Eventually, insects were sorted into various taxonomic groups (to species where possible, but at least to genus or family). We focused on insects in the subcortical community associated with *Juglans* in California (Seybold et al., 2016). After collection at the end of each assay, branch sections were dissected to confirm that no incidental *P. juglandis* had breached the wire mesh screening to colonize and bore in, as the naturally produced male aggregation pheromone released after entry would attract more *P. juglandis* and likely lead to an overestimate of our assessment of intrinsic landing rate.

#### Data handling and statistical analysis

Data from assays comparing *P. juglandis* landing rates on *J. californica* and *J. major* were grouped (assays 1–4). For those species and instances where trap catch counts were relatively high (>20 individuals per 10 acetate sheets), data were analyzed separately by insect species (i.e., *P. juglandis*, *X. saxeseni*, *H. eruditus*, and *N. brevipennis*) and sex (*P. juglandis* and *N. brevipennis*) using a generalized linear mixed model [function glmer.nb() from package lme4 in R; R Core Team, 2016] with location (i.e., NCGR or ATRC) and walnut species as fixed factors. Assay number and sampling period were treated as random factors. The distributions of insects landing on the adhesive-coated acetate sheets were modeled as a negative binomial, and this model was selected because of its superior handling of over-dispersed data (compared to a Poisson distribution). The link function between the mean of the catches and the linear predictor was the default natural logarithm. Since

there was no significant interaction between location and walnut species for either insect species or sex, these interactions were not included in the model.

Data from assays comparing *P. juglandis* landing rates on *J. californica* branch sections and on cardboard tubes (assays 5 and 6) were modeled and analyzed similarly to data from assays 1–4 (see above). Data from assay 6 were analyzed in two stages. The first round compared landing rates on *J. californica* branch sections and on cardboard tubes, whereas the second round compared landing rates on two age classes of *J. californica* branch sections with those on cardboard tubes. In the second round, if the overall null hypothesis (no significant differences among *J. californica*-old, *J. californica*-new, and control) was rejected, then comparisons of the three means were conducted by using the lsmeans() function from the package 'emmeans' (R Core Team, 2016). The significance level for these comparisons was adjusted by Tukey's honestly significant differences (HSD) test.

#### Voucher specimens and nomenclature

Most specimens of Coleoptera of interest were identified by SJ Seybold with consultation with Dr. Donald E. Bright, Jr. (Colorado State University, Fort Collins, CO, USA; retired) for bark and ambrosia beetles, and Richard Westcott (Oregon Department of Agriculture, Salem, OR, USA; retired) for flatheaded borers (Buprestidae). The sexes of *P. juglandis* and *N. brevipennis* were separated based on morphological characters described, respectively, in Seybold et al. (2013b) (pubescence on the female frons and granules on the male elytral declivity) and Linsley (1963) [testaceous (brown) body color and modified and setose second and third abdominal sternites of the female and piceous (black) body color and slender, delicate abdominal form of the male]. Voucher specimens of adults were accessioned into the Entomology Department at the California Academy of Sciences (CAS), San Francisco, CA, and the California State University-Chico Collection, Chico (CHSC). For this study, we have elected to use the original nomenclature for bark and ambrosia beetles (Scolytidae) based on the arguments presented in Bright (2014, 2019). In essence, morphological and fossil evidence of adult scolytids supports the family-level treatment, whereas similarity in scolytid and curculionid larval morphology supports a subfamily placement. Because this issue is not entirely resolved, we prefer to take the more conservative approach of using the original nomenclature. Many green lacewings (Chrysopidae) and brown lacewings (Hemerobiidae, both Neuroptera) were collected in the funnel traps during the surveys at the NCGR and the ATRC. The specimens from the ATRC were identified by Dr. Catherine A. Tauber (UCD). Voucher specimens of these adults were deposited in the UCD Bohart Insect Museum. Similarly, snakeflies (Raphidioptera: Raphidiidae) were collected in the funnel traps and branch section traps at both the NCGR and ATRC. These were identified by Dr. Shaun Winterton, California Department of Food and Agriculture, Sacramento (CA, USA). Voucher specimens of these adults were deposited in the California State Collection of Arthropods.

## Results

#### *Pityophthorus juglandis* seasonal flight monitoring

Weekly trap catches revealed that *P. juglandis* flight activity–abundance was greater at the NCGR than at the ATRC (Figure 4). The seasonal flight data were used to guide the timing of the landing rate assays, which were initiated in spring 2015 (assay 1), fall 2015 (assay 2), spring 2016 (assays 3 and 4), fall 2016 (assay 5), and spring 2019 (assay 6). All assays were conducted during periods of flight when the highest landing rates were anticipated, although assays 2, 4, and 5 tended to miss these windows (Figure 4; Seybold et al., 2012; Chen & Seybold, 2014). Flight peaks at the NCGR with a mean catch greater than 50 *P. juglandis* per trap per day were observed during spring and summer 2015, and during summer and early fall 2018, whereas flight peaks in 2014, 2016, 2017, and 2019 were below 50 *P. juglandis.* At the ATRC, flight peaks with a mean catch greater than 2 *P. juglandis* per trap per day were observed during fall 2015, spring and fall 2016, spring 2017, fall 2018, and spring 2019. At both locations, primarily during flight peaks, twice as many females were trapped than males.

#### *Pityophthorus juglandis* landing rate on walnut branch section traps

In total 1 153 *P. juglandis* (504 males and 649 females) were trapped during assays 1–4 (assay 1: 142 males, 152 females; assay 2: 46 males, 54 females; assay 3: 310 males, 432 females; and assay 4: 6 males, 11 females). Pooled *P. juglandis* landing rates from assays 1–3 (NCGR), and assay 4 (ATRC) suggested host preferences by both male and female *P. juglandis* for *J. californica* over *J. major* (NCGR: n = 230 branch sections; ATRC: n = 100 branch sections; male *P. juglandis*: $\chi^2 = 3.95$, P = 0.046; female *P. juglandis*: $\chi^2 = 6.06$, P = 0.013, both d.f. = 1) (Figure 5). Comparing locations, there was greater male and female *P. juglandis* flight activity–abundance at NCGR than at ATRC (NCGR: n = 230 branch sections; ATRC: n = 100 branch sections; male *P. juglandis*: $\chi^2 = 52.91$; female *P. juglandis*: $\chi^2 = 81.67$, both d.f. = 1, P<0.001).

In total 82 *P. juglandis* (21 males and 61 females) were trapped during assay 5 (NCGR), and *P. juglandis* males and females showed no preference for *J. californica* over the control (cardboard tube) (n = 90 branch sections;

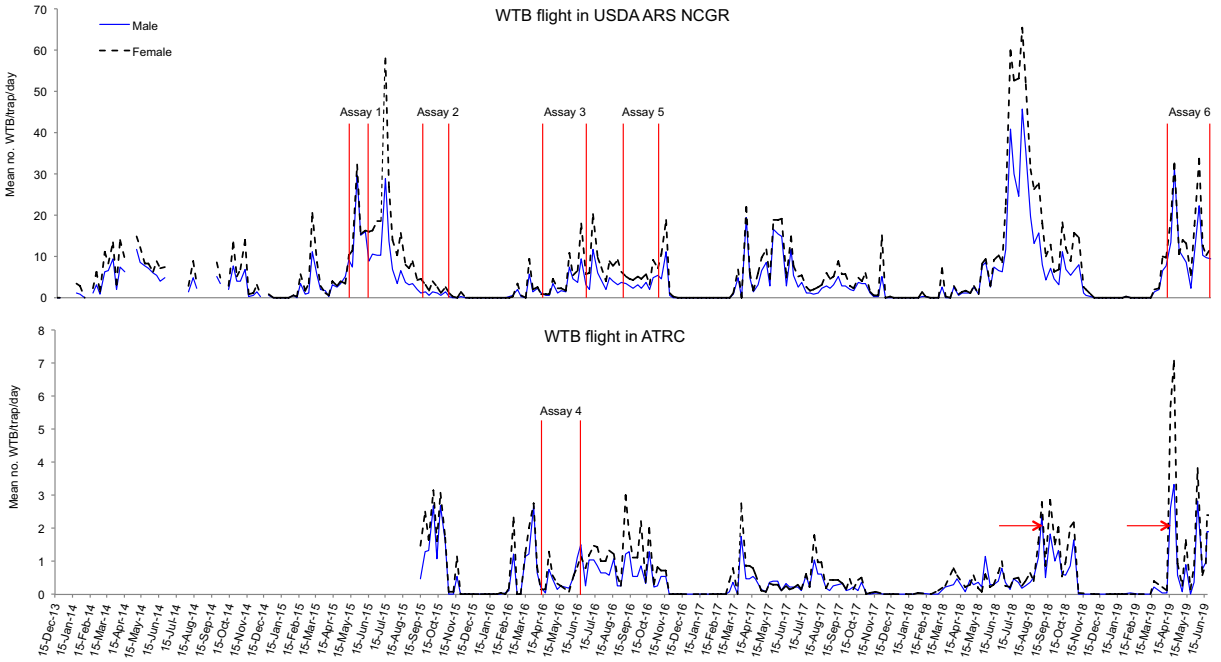


**Figure 4** Walnut twig beetle (WTB), *Pityophthorus juglandis*, flight at the National Clonal Germplasm Repository (NCGR) at Wolfskill Experimental Orchards in Winters, CA, USA (2014–2019) and at the California State University Agricultural Teaching and Research Center (ATRC) in Chico, CA (2015–2019). Note the different scales on the Y-axes. Six flight landing assays were conducted during the various date ranges. Arrows point to the date ranges (4–11 September 2018 and 16–23 April 2019) when individual females of *Hypothenemus eruditus* were trapped at the ATRC. Daily maximum temperatures and precipitation (Winters A., CIMIS weather station no. 139 and Chico C., NCDC weather station no. 1715, CSU Chico University Farm) are: assay 1 (19.4–32.2 °C, 1 mm), assay 2 (20.6–40 °C, 0.3–1.8 mm), assay 3 (17.8–37.2 °C, 0.3–5.6 mm), assay 4 (16.7–38.3 °C, 0.5–16.5 mm), assay 5 (17.2–36.1 °C, 0.3–9.6 mm).

male *P. juglandis*: $\chi^2 = 0.048$, P = 0.83; female *P. juglandis*: $\chi^2 = 0.35$, P = 0.55, both d.f. = 1) (Figure 6). In total 356 *P. juglandis* (156 males, 200 females) were trapped during assay 6 (NCGR) (round 1: 91 males, 120 females; round 2: 65 males, 80 females). In round 1 (Figure 7A), *P. juglandis* males and females showed a preference for *J. californica* over the control (cardboard tube) (n = 50 branch sections; male: $\chi^2 = 5.44$, P = 0.02; female *P. juglandis*: $\chi^2 = 4.45$, P = 0.04, both d.f. = 1). In round 2 (Figure 7B), *P. juglandis* males and females showed no preference among *J. californica*-old, *J. californica*-new, and the control (n = 60 branch sections; male *P. juglandis*: $\chi^2 = 1.55$, P = 0.46; female *P. juglandis*: $\chi^2 = 4.03$, P = 0.13, both d.f. = 2). Dissection of branch sections at the end of all trials revealed no evidence of incidental attacks by *P. juglandis* under the fine mesh metal screening during the course of each assay (i.e., no new entrance/emergence holes, galleries, frass, or live adults).

#### Associated insects and seasonal flight monitoring

At the NCGR, seasonal survey trap catches for *P. juglandis* collected from January 2014 through June 2019 also yielded other herbivorous insects (primarily Coleoptera) or predaceous insects (primarily Neuropteroidea) thought to be associated with *Juglans* or other hardwood trees growing nearby (Table 2). Female *X. saxeseni* were trapped from May to June 2014 (4 specimens), March to June 2015 (8), March to September 2016 (22), February to August 2017 (10), March to June 2018 (9), and March to June 2019 (33). Two cerambycid beetle species were trapped during this survey: *Phymatodes juglandis* Leng (one specimen, 25 March to 2 April 2018) and *Xylotrechus nauticus* (Mannerheim), the latter of which was detected from March to July 2015 (11), April 2016 (1), May to July 2017 (2), May to August 2018 (7), and in June 2019 (2). *Hypothenemus eruditus* was trapped during 15–22 September 2014 (1), July to August 2015 (2), April to September 2016 (7), May to October 2017 (24), February to November 2018 (21), and April to June 2019 (9). The western oak bark beetle, *Pseudopityophthorus pubipennis* LeConte, was trapped in small numbers, April to May 2015 (2), April to June 2016 (2), July 2017 (2), and August 2018 (1), as was the shothole borer, *Scolytus rugulosus* (Müller) (both Scolytidae), which appeared in survey traps during March

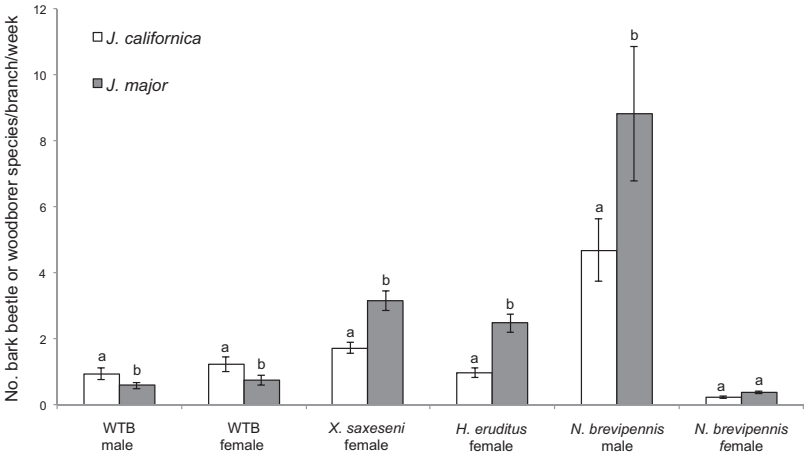


**Figure 5** Mean (± SE) weekly landing rates (no. specimens per branch per week) of male and female walnut twig beetle (WTB), *Pityophthorus juglandis* (n = 330), female *Xyleborinus saxeseni* (n = 330), female *Hypothenemus eruditus* (n = 230), and male and female *Nathrius brevipennis* (n = 330) on adhesive-coated acetate sheets surrounding branch sections of *Juglans californica* and *J. major* (n = 330). Landing rates from four assays were combined (assays 1–4, see Figure 4 for date ranges). Assays 1–3 were conducted at the National Clonal Germplasm Repository (NCGR), assay 4 was conducted at the California State University Chico Agricultural Teaching and Research Center (ATRC). Weekly landing rates of female *Hypothenemus eruditus* were only recorded at the NCGR (assays 1–3, n = 230). Different letters capping means within a species × sex combination indicate a significant difference between walnut species (lsmeans: $P<0.05$).

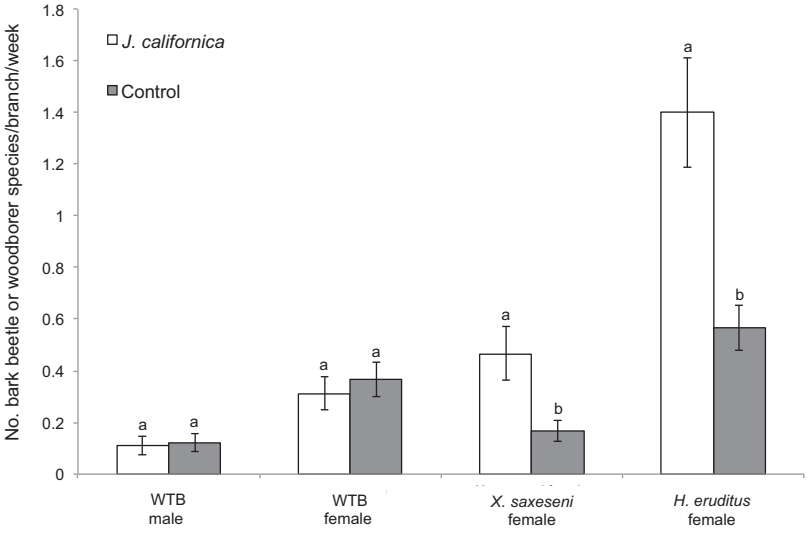


**Figure 6** Mean (± SE) weekly landing rates (no. specimens per branch per week) of male and female walnut twig beetle (WTB), *Pityophthorus juglandis*, female *Xyleborinus saxeseni*, and female *Hypothenemus eruditus* on adhesive-coated acetate sheets surrounding branch sections of *Juglans californica* and cardboard tubes (control) (n = 90). Landing rate assay 5 was conducted at the National Clonal Germplasm Repository (NCGR). Different letters capping means within a species × sex combination indicate a significant difference between *J. californica* and the negative control (lsmeans: $P<0.05$).

to November 2014 (4), April to August 2015 (4), April to June 2017 (3), April to July 2018 (8), and April to June 2019 (11). None of the latter were observed in 2016. A scolytid species that was unexpectedly present at the NCGR (and later at the ATRC) was the ambrosia beetle *Xyleborus affinis* Eichhoff. This species is native to eastern North America and Central and South America, but one specimen was trapped at the NCGR in a survey trap in March 2015. Two woodborer species were caught: the lead cable borer, *Scobicia declivis* LeConte (Bostrichidae), was active

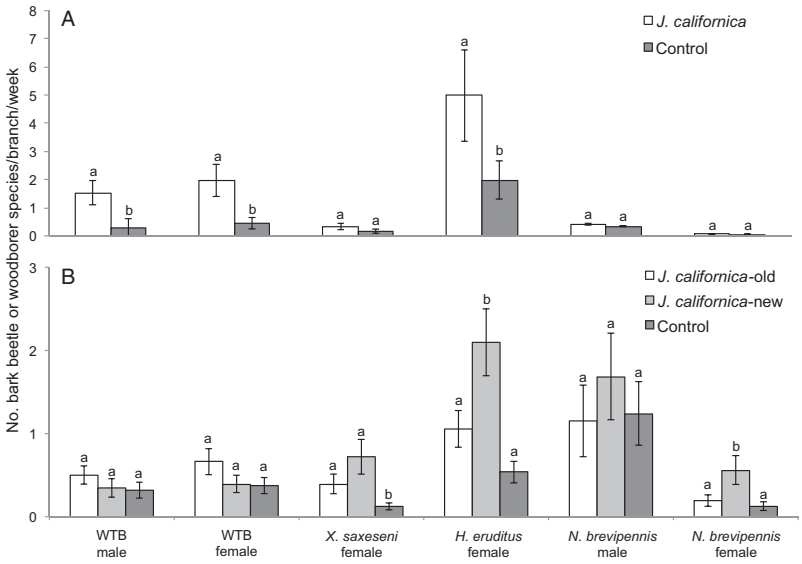


**Figure 7** Mean (± SE) weekly landing rates (no. specimens per branch per week) of male and female walnut twig beetle (WTB), *Pityophthorus juglandis*, female *Xyleborinus saxeseni,* female *Hypothenemus eruditus,* and male and female *Nathrius brevipennis* on adhesive-coated acetate sheets surrounding branch sections of (A) *Juglans californica* and cardboard tubes (control) (n = 50), and (B) *J. californica*-old (i.e., branch sections that had been present in the field for 5 weeks), *J. californica*-new (i.e., branch sections newly placed from freezer storage in week 6), and cardboard tubes (control) (n = 60). Ten pairs of old and new branch sections originated from the same trees in the native range of *J. californica* (Table S2). Landing rate assay 6 was conducted at the National Clonal Germplasm Repository (NCGR). Different letters capping means within a panel indicate significant differences between treatments (lsmeans, adjusted by Tukey's HSD test: $P<0.05$).

in November 2014 (1), July 2015 (1), March to May 2016 (2), April to August 2017 (2), and April to September 2018 (5), and *Polycesta californica* LeConte (Buprestidae) was active in May 2014 (3), May to June in 2015 and 2016 (9 total), May 2018 (3), and June 2019 (3). None of the latter were observed in 2017. This buprestid is common in California and Oregon and recorded hosts include species of the genera *Alnus*, *Acer*, *Arbutus*, *Quercus*, *Populus*, *Cercocarpus*, and *Salix* (Furniss & Carolin, 1977; MacRae, 2006).

Predaceous lacewings, Chrysopidae and Hemerobiidae (both Neuroptera), were trapped at NCGR throughout each year from 2014 to 2019 (252 Chrysopidae and eight Hemerobiidae); however, they were not identified to species. Predaceous snakeflies, *Agulla* sp. (Raphidiidae), were also trapped at the NCGR from 2014 to 2019 (March to June) with a total of 346 (72 males, 93 females, 181 not identified to sex). From this catch, six males and six females (caught in April 2015) were identified as *Agulla modesta* Carpenter. The remaining were not identified to species.

At the ATRC, seasonal survey trap catches included female *X. saxeseni* from February to June 2016 and March to June 2017 (at low abundance, <10 per year); and from March to June 2019 (at intermediate abundance, <40 per year) (Table 3). They also included male and female *N. brevipennis* from April to May 2016, May to June 2017, May to July 2018, and April to June 2019 (at intermediate abundance, <50 per year). In 3 of 4 years more males were trapped than females (Table 3). Another longhorned beetle, *Stenocorus nubifer* (LeConte) (Cerambycidae), was trapped only at the ATRC in seasonal survey traps in April and May during 2016, 2017, 2018, and 2019, corresponding with the flight peak (April to July) described by Linsley & Chemsak (1972). This insect was not trapped during the NCGR seasonal survey or on the adhesive-coated acetate sheets from landing rate studies at either site. Its larval host plant in or around the ATRC is not known. In seasonal survey trap catches at the ATRC, one specimen of *P. californica* was trapped in late May 2016, whereas two female specimens of *H. eruditus* were trapped in September 2018 and in April 2019, respectively (Figure 4). One male *S. rugulosus* was also trapped in an ATRC flight survey trap in April 2018. Thus, in most instances the flight activity–abundance of these subcortical insect associates of *P. juglandis* was relatively low at the ATRC.

Three species of Chrysopidae and one species of Hemerobiidae – *Chrysopa quadripunctata* Burmeister, *Chrysoperla comanche* (Banks), *Chrysoperla carnea* (Stephens)

**Table 2** Subcortical insect associates and predators of walnut twig beetle, *Pityophthorus juglandis*, trapped during flight monitoring at the USDA ARS National Clonal Germplasm Repository (NCGR) with maximum periods of annual flight (2014–2019)

| | | | | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | | 2019 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Order | Family | Species | Sex | No. | Flight range | No. | Flight range | No. | Flight range | No. | Flight range | No. | Flight range | No. | Flight range |
| Coleoptera | Bostrichidae | *Scobicia declivis* LeConte | | 1 | Nov | 1 | Jul | 2 | May | 2 | Apr–Aug | 5 | Apr–Sep | 0 | – |
| | Buprestidae | *Polycesta californica* LeConte | | 3 | May | 3 | May–Jun | 6 | May–Jun | 0 | – | 3 | May | 3 | Jun |
| | Cerambycidae | *Nathrius brevipennis* (Mulsant) | Male | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – | 2 | May–Jun |
| | | | Female | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – |
| | | *Stenocorus nubifer* (LeConte) | | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – |
| | | *Xylotrechus nauticus* (Mannerheim) | | 0 | – | 11 | Mar–Jul | 1 | Apr | 2 | May–Jul | 7 | May–Aug | 2 | Jun |
| | Scolytidae | *Hypothenemus eruditus* Westwood | | 1 | Sep | 2 | Jul–Aug | 7 | Apr–Sep | 24 | May–Oct | 21 | All year | 9 | Apr–Jun |
| | | *Pseudopityophthorus pubipennis* LeConte | | 0 | – | 0 | – | 2 | Apr–Jun | 0 | – | 0 | – | 0 | – |
| | | *Scolytus rugulosus* (Müller) | | 4 | Mar–Nov | 4 | Apr–Aug | 0 | – | 3 | Apr–Jun | 8 | Apr–Jul | 11 | Apr–Jun |
| | | *Xyleborinus saxeseni* (Ratzeburg) | | 4 | May–June | 8 | Mar–Jun | 22 | Mar–Sep | 10 | Feb–Aug | 9 | Mar–Jun | 33 | Mar–Jun |
| | | *Xyleborus affinis* Eichhoff | | 0 | – | 1 | Mar | 0 | – | 0 | – | 0 | – | 0 | – |
| Neuroptera | Chrysopidae | *Chrysopa carnea* (Stephens) species group | | – | – | – | – | – | – | – | – | – | – | – | – |
| | | *Chrysopa comanche* (Banks) | | – | – | – | – | – | – | – | – | – | – | – | – |
| | | *Chrysopa quadripunctata* Burmeister | | – | – | – | – | – | – | – | – | – | – | – | – |
| | | *Chrysopa* sp. | | 31 | All year | 25 | All year | 36 | All year | 17 | All year | 68 | All year | 75 | All year |
| | Hemerobiidae | *Hemerobius ovalis* Carpenter | | – | – | – | – | – | – | – | – | – | – | – | – |
| | | *Hemerobius pacificus* Banks | | – | – | – | – | – | – | – | – | – | – | – | – |
| | | *Hemerobius* sp. | | 2 | May | 2 | Mar | 1 | Aug | 1 | Oct | 1 | May | 1 | Jun |
| | | *Sympherobius perparvus* McLachlan | | – | – | – | – | – | – | – | – | – | – | – | – |
| Raphidioptera | Raphidiidae[1] | *Agulla modesta* | Male | – | – | 6 | April | – | – | – | – | – | – | – | – |
| | | | Female | – | – | 6 | April | – | – | – | – | – | – | – | – |
| | | *Agulla* sp. | Male | 7 | Mar–Jun | 26 | Mar–Jun | 12 | Apr–May | 16 | Mar–May | 0 | – | 5 | May–Jun |
| | | | Female | 11 | Mar–Jun | 33 | Mar–Jun | 17 | Apr–May | 24 | Mar–May | 2 | Mar–Apr | 0 | – |

–, not listed/identified.

[1]Specimens for 2014 (2 total) and 2015 (179 total) missing and therefore not listed/identified.

**Table 3** Subcortical insect associates and predators of walnut twig beetle, *Pityophthorus juglandis,* trapped during flight monitoring at the California State University Chico, Agricultural Teaching and Research Center (ATRC) with maximum periods of annual flight (2015–2019)

| | | | | 2015 | | 2016 | | 2017 | | 2018 | | 2019 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Order | Family | Species | Sex | No. | Flight range | No. | Flight range | No. | Flight range | No. | Flight range | No. | Flight range |
| Coleoptera | Bostrichidae | *Scobicia declivis* LeConte | | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – |
| | Buprestidae | *Polycesta californica* LeConte | | 0 | – | 1 | May–Jun | 0 | – | 0 | – | 0 | – |
| | Cerambycidae | *Nathrius brevipennis* (Mulsant) | Male | 0 | – | 20 | April–Jun | 15 | May–Jun | 30 | May–Jul | 27 | Apr–Jun |
| | | | Female | 0 | – | 3 | May–Jun | 22 | May–Jun | 24 | May–Jul | 14 | Apr–Jun |
| | | *Stenocorus nubifer* (LeConte) | | 0 | – | 19 | Apr–May | 74 | May | 5 | Apr–May | 13 | Apr–May |
| | | *Xylotrechus nauticus* (Mannerheim) | | 0 | – | 0 | – | 3 | May | 1 | Apr | 1 | Jun |
| | Scolytidae | *Hypothenemus eruditus* Westwood | | 0 | – | 0 | – | 0 | – | 1 | Sep | 1 | Apr |
| | | *Pseudopityophthorus pubipennis* LeConte | | 0 | – | 0 | – | 0 | – | 0 | – | 0 | – |
| | | *Scolytus rugulosus* (Müller) | | 0 | – | 0 | – | 0 | – | 1 | Apr | 0 | – |
| | | *Xyleborinus saxeseni* (Ratzeburg) | | 0 | – | 8 | Feb–Jun | 7 | Mar–Jun | 0 | – | 39 | Mar–Jun |
| Neuroptera | Chrysopidae | *Chrysopa carnea* (Stephens) species group | | 0 | – | 17 | All year | 20 | All year | 28 | All year | 3 | Jan |
| | | *Chrysopa comanche* (Banks) | | 6 | Sep–Dec | 22 | All year | 5 | Mar–Jun | 31 | All year | – | – |
| | | *Chrysopa quadripunctata* Burmeister | | 1 | Sep | 0 | – | 0 | – | 0 | – | – | – |
| | | *Chrysopa* sp. | | 0 | – | 0 | – | 0 | – | 0 | – | 19 | All year |
| | Hemerobiidae | *Hemerobius ovalis* Carpenter | | 0 | – | 0 | – | 0 | – | 4 | May–Jul | – | – |
| | | *Hemerobius pacificus* Banks | | 0 | – | 6 | May–Oct | 6 | Feb–Jul | 3 | Jan | – | – |
| | | *Hemerobius* sp. | | 0 | – | 0 | – | 0 | – | 0 | – | 6 | Jan |
| | | *Sympherobius perparvus* McLachlan | | 0 | – | 0 | – | 0 | – | 1 | Apr | – | – |
| Raphidioptera | Raphidiidae | *Agulla modesta* | Male | – | – | – | – | – | – | – | – | – | – |
| | | | Female | – | – | – | – | – | – | – | – | – | – |
| | | *Agulla* sp. | Male | 0 | – | 5 | Apr–Jun | 2 | May–Jun | 2 | May–Jun | 0 | – |
| | | | Female | 0 | – | 16 | Apr–Jun | 8 | May–Jun | 5 | May–Jun | 0 | – |

–, not listed/identified.

species group (one or more of several possible species including *C. adamsi*, *C. johnsoni*, and *C. plorabunda*), and *Hemerobius pacificus* Banks – were trapped in the 2015–2018 ATRC seasonal survey traps. In total 64 specimens of the green lacewing *C. comanche* were trapped (maximum flight activity in the fall), whereas 65 specimens from the *C. carnea* species group and 15 specimens of *H. pacificus* were also trapped (maximum flight activity in the spring for these latter two taxa). Only one specimen of *C. quadripunctata* (a female) was trapped, and it was caught in the fall. For *C. comanche*, *C. carnea* group, and *H. pacificus*, more females were trapped than males. This difference was more pronounced with *C. comanche* (15 males and 46 females, three indeterminate sex) and the *C. carnea* group (22 males and 43 females), than with *H. pacificus* (six males and nine females). In 2018, two additional species of Hemerobiidae were trapped, *Hemerobius ovalis* (two males, two females) and *Sympherobius perparvus* (one male). In 2019, six Hemerobiidae were trapped but not identified to species. In 2019, 22 Chrysopidae were trapped and from this catch three females were identified to the *C. carnea* group. These insects may function as predators of *P. juglandis* in walnut orchards or native walnut stands.

In total 38 *Agulla* sp. were also trapped at the ATRC from 2016 to 2018 (no specimens were trapped during 2015 and 2019). Five males and 16 females were trapped from April to June in 2016, whereas two males and eight females were trapped from May to June in 2017, and two males and five females were trapped from May to June in 2018.

#### Associated insect landing rate on walnut branch section traps

In addition to *P. juglandis*, trap catches on sticky traps in the various assays yielded other insects thought to be associated with *P. juglandis* on *Juglans* or perhaps directly with other hardwood trees growing nearby (Table 4). These included *Petalium californica* Fall, *Priobium punctatum* LeConte, and *Ptilinus* sp. Müller (all Anobiidae), *S. declivis* (Bostrichidae), *N. brevipennis* (Cerambycidae), *Stenomimus* sp. (Curculionidae), and *Cyclorhipidion bodoanum* (Reitter), *H. eruditus*, *P. pubipennis*, *S. rugulosus*, *X. affinis*, and *X. saxeseni* (all Scolytidae) (Seybold et al., 2016). Predaceous insects trapped in the assays included two species of Laemophloeidae (Coleoptera) – *Narthecius simulator* Casey and *Parandrita cephalotes* (LeConte) (Seybold et al., 2016) – and Neuropteroidea, which were not recovered from the traps due to their generally damaged condition after contacting the trap adhesive. Two probable parasitoids of *P. juglandis* collected in the assays were *Plastonoxus westwoodi* Kieffer (Bethylidae) and *Neocalasoter pityophthori* (Ashmead) (Pteromalidae, both Hymenoptera) (Seybold et al., 2016). A third parasitoid trapped in relatively large numbers in our assays – *Gildoria* sp. (Hymenoptera: Braconidae, Doryctinae) – is likely associated with *N. brevipennis* based on co-rearing data from *J. regia* twigs (Lona, 2019).

In total 1 606 female *X. saxeseni* were trapped at both the NCGR and ATRC during assays 1–4 (assay 1: 301; assay 2: 33; assay 3: 985; assay 4: 287), that is, flight was primarily in the spring and early summer. Males of this species are flightless. Pooled results from assays 1–3 (NCGR) and assay 4 (ATRC) showed that female *X. saxeseni* preferred to land on *J. major* branch section traps over those of *J. californica* (NCGR: n = 230 branch sections; ATRC: n = 100 branch sections; $\chi^2 = 25.78$, d.f. = 1, $P<0.001$) (Figure 5). In these assays, the flight activity–abundance of female *X. saxeseni* at the NCGR was greater than at the ATRC (NCGR: n = 230 branch sections; ATRC: n = 100 branch sections; $\chi^2 = 5.86$, d.f. = 1, $P = 0.015$). In total 57 female *X. saxeseni* were trapped during assay 5 (NCGR). In this assay, female *X. saxeseni* preferred to land on *J. californica* branch section traps vs. the cardboard tube controls (n = 90 branch sections; $\chi^2 = 5.86$, d.f. = 1, $P = 0.015$) (Figure 6). In total 94 (round 1: 25; round 2: 69) female *X. saxeseni* were trapped during assay 6 (NCGR). In round 1 of this assay (Figure 7A), female *X. saxeseni* showed no preference for landing on *J. californica* branch section traps vs. the cardboard tube controls (n = 50 branch sections; $\chi^2 = 2.17$, d.f. = 1, $P = 0.14$). In round 2 (Figure 7B), female *X. saxeseni* showed a preference (n = 60 branch sections; $\chi^2 = 19.31$, d.f. = 2, $P<0.001$), and they preferred to land on both *J. californica* treatments over the cardboard tube controls (*J. californica*-old vs. *J. californica*-new: $Z = 2.20$, $P = 0.07$; *J. californica*-old vs. control: $Z = 2.62$, $P = 0.02$; *J. californica*-new vs. control: $Z = 4.22$, $P<0.001$).

During comparisons of landing rates on *J. californica* vs. *J. major*, in total 789 female *H. eruditus* were trapped at the NCGR (assay 1: 6; assay 2: 226; assay 3: 557), whereas none were trapped at the ATRC (assay 4). Similar to *X. saxeseni*, *H. eruditus* males are flightless. Pooled results from assays 1–3 (NCGR) showed that female *H. eruditus* preferred to land on *J. major* branch section traps over those of *J. californica* (n = 230 branch sections; $\chi^2 = 38.18$, d.f. = 1, $P<0.001$) (Figure 5). In total 177 female *H. eruditus* were trapped during assay 5 (NCGR). In this assay, female *H. eruditus* preferred landing on *J. californica* branch section traps vs. the cardboard tube controls (n = 90 branch sections; $\chi^2 = 19.08$, d.f. = 1, $P<0.001$) (Figure 6). In total 554 (round 1: 348; round 2: 206) female *H. eruditus* were trapped during assay 6 (NCGR). In round 1 of this assay (Figure 7A), female *H. eruditus* showed a significant tendency to land on

**Table 4** Landing rates (mean no./branch/week) of insects associated with *Pityophthorus juglandis*, *Juglans* sp., and other hardwood trees on acetate sticky sheets surrounding branch sections of *Juglans californica* (CABW), *J. major* (AZBW), or a cardboard tube (control) for assays 1–6, Solano and Butte County, CA, USA

| | | | | Assay 1 | | Assay 2 | | Assay 3 | | Assay 4 | | Assay 5 | | Assay 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Order | Family | Species | Sex | CABW | AZBW | CABW | AZBW | CABW | AZBW | CABW | AZBW | CABW | Control | CABW-old | CABW-new | Control |
| Coleoptera | Anobiidae | *Petalium californica* Fall | | 0.08 | 0.13 | 0.01 | 0 | 1.22 | 0.78 | 0.02 | 0 | 0 | 0 | – | – | – |
| | | *Priobium punctatum* LeConte | | 0.63 | 0.48 | 0 | 0 | 1.16 | 1.33 | 0.17 | 0.07 | 0 | 0 | – | – | – |
| | | *Ptilinus* sp. | | 0.05 | 0.15 | 0 | 0 | 0.02 | 0.01 | 0.01 | 0.01 | 0 | 0.01 | – | – | – |
| | Bostrichidae | *Scobicia declivis* LeConte | | 0.08 | 0.15 | 0.20 | 0.29 | 0.25 | 0.32 | 0.02 | 0 | 0 | 0 | 0.08 | 0 | 0.02 |
| | Cerambycidae | *Nathrius brevipennis* (Mulsant) | Male | 1.95 | 0.50 | 0 | 0 | 3.73 | 5.98 | 10.56 | 22.34 | 0 | 0 | 0.75 | 1.57 | 0.78 |
| | | | Female | 0.08 | 0.10 | 0 | 0 | 0.40 | 0.55 | 0.28 | 0.57 | 0 | 0 | 0.13 | 0.52 | 0.08 |
| | Laemophloeidae | *Narthecius simulator* Casey | | 0.10 | 0.13 | 0.01 | 0.01 | 0.15 | 0.05 | 0.04 | 0.04 | 0.01 | 0.03 | 0.05 | 0.07 | 0.04 |
| | | *Parandrita cephalotes* (LeConte) | | 0.33 | 0.58 | 0 | 0 | 0.11 | 0.15 | 0 | 0 | 0 | 0 | 0.08 | 0.13 | 0.09 |
| | Curculionidae | *Stenomimus* sp. | | – | – | – | – | – | – | – | – | 0.03 | 0 | 0.03 | 0.02 | 0.05 |
| | Scolytidae | *Cyclorhipidion bodoanum* (Reitter) | | 0.05 | 0.10 | 0 | 0 | 0.04 | 0.03 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0.02 |
| | | *Hypothenemus eruditus* Westwood | | 0.05 | 0.10 | 0.74 | 2.09 | 1.45 | 3.61 | 0 | 0 | 1.40 | 0.57 | 2.77 | 2.10 | 1.17 |
| | | *Pseudopityophthorus pubipennis* LeConte | | 0.03 | 0.08 | 0 | 0 | 0.02 | 0.01 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 |
| | | *Scolytus rugulosus* (Müller) | | 0.08 | 0.15 | 0.05 | 0.03 | 0.23 | 0.15 | 0 | 0 | 0.01 | 0.01 | 0.15 | 0.20 | 0.27 |
| | | *Xyleborus affinis* Eichhoff | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0 |
| | | *Xyleborinus saxeseni* (Ratzeburg) | | 1.75 | 5.78 | 0.10 | 0.31 | 3.58 | 5.37 | 0.96 | 1.91 | 0.47 | 0.17 | 0.35 | 0.67 | 0.14 |
| Hymenoptera | Bethylidae | *Plastonoxus westwoodi* Kieffer | | 0.45 | 0.40 | 0.19 | 0.15 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Braconidae | *Gildoria* sp. | | – | – | – | – | 0.45 | 0.34 | 0.89 | 0.76 | 0.01 | 0.03 | 0.05 | 0.10 | 0.02 |
| | Pteromalidae | *Neocalosoter pityophthori* (Ashmead) | | 0.35 | 0.30 | 0.51 | 0.60 | 0.20 | 0.40 | 0.01 | 0.02 | 0.44 | 0.32 | 0 | 0 | 0.01 |

Assays were conducted during the following date ranges: assay 1 (5 May to 6 June 2015, n = 40); assay 2 (29 August to 24 October 2015, n = 80); assay 3 (10 April to 24 June 2016, n = 110); assay 4 (13 April to 21 June 2016, n = 100); assay 5 (27 August to 29 October 2016, n = 90); assay 6 (8 April to 21 June 2019, n = 110; CABW-new only 13 May to 21 June 2019, n = 60).

–, not listed/identified.

*J. californica* branch section traps over cardboard tubes (negative control) (n = 50 branch sections; $\chi^2 = 19.41$, d.f. = 1, P<0.001). In round 2 (Figure 7B), female *H. eruditus* showed a significant preference (n = 60 branch sections; $\chi^2 = 25.84$, d.f. = 2, P<0.001), and they preferred to land on *J. californica*-new over *J. californica*-old and the cardboard tube control (*J. californica*-old vs. *J. californica*-new: Z = 2.85, P = 0.01; *J. californica*-old vs. control: Z = 2.32, P = 0.053; and *J. californica*-new vs. control: Z = 4.98, P<0.001).

In total 4 653 *N. brevipennis* (4 457 males and 196 females) were trapped during assays 1–4 (assay 1: 99 males, seven females; assay 2: 0 males, 0 females; assay 3: 1 068 males, 104 females; assay 4: 3 290 males, 85 females). Overall, *N. brevipennis* sex ratio in these trials ranged from 10:1 (assay 3) to 39:1 (assay 4), that is, more males than females were caught (Figure 5). Pooled results from assays 1–3 (NCGR) and assay 4 (ATRC) suggested that male *N. brevipennis* preferred to land on *J. major* branch section traps, whereas females showed no significant host preference (NCGR: n = 230 branch sections; ATRC: n = 100 branch sections; males: $\chi^2 = 7.25$, P = 0.007; females: $\chi^2 = 2.86$, P = 0.09, both d.f. = 1) (Figure 5). There was no difference in flight activity–abundance of male and female *N. brevipennis* during the landing assays at the NCGR and ATRC (NCGR: n = 230 branch sections; ATRC: n = 100 branch sections; males: $\chi^2 = 0.002$, P = 0.96; females: $\chi^2 = 1.92$, P = 0.17, both d.f. = 1). No comparison between *N. brevipennis* landing rates on *J. californica* vs. control were carried out for assay 5 at the NCGR, as this species apparently flies during the spring and early summer and not during the fall (our data; Linsley, 1963). However, in assay 6 (spring 2019, NCGR), in total 319 *N. brevipennis* (265 males and 54 females; sex ratio 5:1) were trapped (round 1: 38 males, five females; round 2: 227 males, 49 females) (Figure 7). In round 1 of this assay (Figure 7A), male and female *N. brevipennis* showed no preference for landing on *J. californica* branch sections over cardboard tubes (negative control) (n = 50 branch sections; male *N. brevipennis*: $\chi^2 = 0.15$, P = 0.70; female *N. brevipennis*: $\chi^2 = 0.21$, P = 0.65, both d.f. = 1). In round 2 (Figure 7B), male *N. brevipennis* showed no preferences among *J. californica*-old, *J. californica*-new, or the cardboard tube (n = 60 branch sections; $\chi^2 = 1.25$, d.f. = 2, P = 0.54). However, female *N. brevipennis* showed a preference (n = 60 branch sections; $\chi^2 = 17.53$, d.f. = 2, P<0.001), and they preferred to land on *J. californica*-new branch section traps over those for *J. californica*-old and the cardboard tube (*J. californica*-old vs. *J. californica*-new: Z = 2.95, P = 0.01; *J. californica*-old vs. control: Z = 0.93, P = 0.62; and *J. californica*-new vs. control: Z = 3.55, P = 0.001).

Seventeen other subcortical insects associated putatively with *Juglans*, other hardwood trees, or *P. juglandis* (Seybold et al., 2016) were trapped during the assays (Table 4). Among the anobiids, *Pr. punctatum* was not caught during assays 2 and 5 at the NCGR, suggesting that it does not fly in the fall. *Petalium californica* was present in both locations and all assays except for assay 5. *Ptilinus* sp. was not caught during assay 2 at the NCGR. *Petalium californica*, *Pr. punctatum*, and *Ptilinus* sp. were present in the trap catches from assay 6 but were not quantified due to time constraints. *Scobicia declivis* was also present in both locations and all assays except for assay 5. *Narthecius simulator*, the *P. juglandis* predator, was trapped in all assays, but a related predator, *P. cephalotes*, was not trapped during assays 2 and 5 at the NCGR or during assay 4 at the ATRC. A *Stenomimus* sp. weevil was trapped at the NCGR during assays 5 (three specimens) and 6 (nine specimens, all during round 2), but was not identified and recorded during assays 1–4. Bark and ambrosia beetles trapped at the NCGR included *C. bodoanum*, *P. pubipennis*, and *S. rugulosus*. *Cyclorhipidion bodoanum* and *P. pubipennis* were not trapped during the fall at the NCGR. *Scolytus rugulosus*, likely associated with neighboring almond orchards, was trapped at low levels in all assays at the NCGR (assay 1: nine specimens; assay 2: six specimens; assay 3: 41 specimens; assay 5: two specimens; assay 6: 58 specimens). One specimen of *X. affinis* was trapped in 2016 at the ATRC landing on a *J. major* branch section during assay 4 (Table 4). A second specimen was caught in 2018 in an unrelated study conducted at the ATRC. Of potential *P. juglandis* parasitoids, *N. pityophthori* was trapped in all assays, but *P. westwoodi* was not caught during assay 4 at the ATRC, not during assays 5 and 6 at the NCGR. *Gildoria* sp., the *N. brevipennis* parasitoid, was present in both locations in assays 3–6. However, its presence during assays 1 and 2 is unknown because we were not aware of this parasitoid when the data were collected.

## Discussion

Seasonal monitoring of trap catches at NCGR (2013–2019) and ATRC (2015–2019) showed that *P. juglandis* flight peaked in late spring/early summer and again in the fall, similar to flight patterns described at two northern California sites by Seybold et al. (2012) and Chen & Seybold (2014). *Pityophthorus juglandis* trap catches at both sites can be used to compare and predict *P. juglandis* abundance in future studies based on population modeling (Chen & Seybold, 2014).

Assays of *P. juglandis* landing rate showed that branch sections of *J. californica* elicited a higher landing rate by *P. juglandis* than branch sections of *J. major*. This is

consistent with the work of Hishinuma (2017), who found that *P. juglandis* discriminated among various *Juglans* spp. by landing on live branches on trees and on branch sections of certain species baited with the aggregation pheromone. Specifically, Hishinuma (2017) reported that *P. juglandis* showed a higher preference for landing on pheromone-baited *J. californica* over *J. major*. The attraction of *P. juglandis* to *unbaited J. californica* branch sections in our work (or relative repellency to *unbaited J. major* branch sections) indicates that *P. juglandis* host preference may be influenced by long-range olfactory cues emitted by the host. Balanced sex ratios in the responses in our assays are consistent with the absence of volunteer attacks by male *P. juglandis* under the mesh metal screening, and indicate that the landing rate was not influenced by naturally produced aggregation pheromone, which tends to attract more females than males (Chen & Seybold, 2014). Furthermore, although we selected branches of *J. californica* that showed little evidence of prior colonization by *P. juglandis* and potentially biased this treatment toward host phenotypes that might be less preferred by *P. juglandis* in our experiments, the results of assays 1–3 and 6 demonstrated that the beetle was still capable of locating and landing on the experimental branch sections of *J. californica*.

Surprisingly, *P. juglandis* showed no preference for landing on *J. californica* over *J. major* branch sections in assay 4 at the ATRC. However, the low flight rate (17 beetles total) of *P. juglandis* at the ATRC (compared to the NCGR) may have precluded the capacity for us to measure any discrimination among the treatments. This emphasizes the need for a sufficiently high *P. juglandis* flight activity–abundance in an orchard or other habitat to conduct studies of intrinsic landing rate (i.e., in the absence of aggregation pheromone). Even under conditions of high population density, landing rates in response to host volatiles alone are low, which may demand that researchers conduct multiple assays or use greater replication to accumulate enough data for statistical analysis.

Several factors may explain the low population density of *P. juglandis* that we noted at the ATRC relative to the density at the NCGR (assay 3 vs. assay 4). The walnut orchard at the ATRC, as well as nearby orchards, is a commercial-style planting of *J. regia*. In contrast, the orchard at the NCGR is comprised of at least 15 species of *Juglans*, and the assays were positioned under the crowns of trees of northern California black walnut, *J. hindsii*, planted as a block in the northeastern corner of that collection. Adjacent to the NCGR is the Putah Creek riparian area, which includes a large number of native *J. hindsii* dying from TCD. Hishinuma (2017) reported that in several assays *J. regia* was less attractive to *P. juglandis* than black walnuts such as *J. californica*, *J. hindsii*, and *J. nigra*. The presence of these latter host species at the NCGR and the greater rate of *P. juglandis* reproduction in these hosts (Hefty et al., 2018) may explain the higher number of *P. juglandis* caught in our survey traps and assays at the NCGR. As the branch sections for assays 3 and 4 were not cut from one accession each for *J. californica* and *J. major*, another possible factor influencing *P. juglandis* landing rates may be the differing branch sources with potential variability in genetically based, intraspecific volatile profiles. Also, wounding or stress cues (e.g., volatiles) of the individual cut branches due to handling, which includes cutting, freezing, and thawing prior to their placement in the field, may have influenced the landing rate. During the assays, volatile composition emitted from the cut branches may have changed during the course of each assay, mainly for assays 3, 4, and 6, which lasted 10–11 weeks. Trap catches of *P. juglandis*, *X. saxeseni*, and *N. brevipennis* were low during the beginning and end of each of these assays but were largest during the middle of the assays (assay 6 had an exceptionally large catch during the 2nd week, but otherwise followed this trend). These trap catches could have been explained by changing volatile release patterns from the branch sections or by changing seasonal flight rhythms of the taxa.

In assay 5, *P. juglandis* showed no preference for *J. californica* over the control (i.e., cardboard mailing tube). *Pityophthorus juglandis* seasonal flight monitoring at both study sites indicated a reduction in flight activity at the time of assay 5, which likely resulted in low rates and minimal data collection during that landing assay. In contrast, assay 6 was conducted at a point in the season when flight activity–abundance was relatively high, and landing rates were perhaps more readily assessed. This assay suggested that *P. juglandis* uses olfactory cues to distinguish a host from a non-host cardboard tube (control), but the relative freshness of the branch sections did not appear to impact the landing rate. Indeed, the older branch sections appeared to be quantitatively more attractive than the newer branch sections.

Other subcortical insects associated with *Juglans* may also use long-range olfactory cues to locate their hosts. Branch sections of *J. major* elicited higher landing rates by female *X. saxeseni* and *H. eruditus* than branch sections of *J. californica* (assays 1–4 and 1–3, respectively). However, in assays 5 and 6, there was a tendency for both invasive species to prefer *J. californica* over the cardboard tube (control). Slightly more *X. saxeseni* were trapped during assay 6 (spring) than during assay 5 (fall). The seasonal trapping surveys at the ATRC and the NCGR suggested that this species was active in flight primarily during the spring, but these traps, baited with the aggregation

pheromone of *P. juglandis*, may not necessarily be an effective tool for establishing the seasonal flight response of *X. saxeseni*. Nonetheless, synthesis of results from all assays suggests that this insect may also use long-range olfactory signals to find hosts. This invasive ambrosia beetle bores into the xylem of hardwoods and conifers (Seybold et al., 2016) and is very responsive to ethanol, which may have emanated from the branch sections over time. Surprisingly the new branch sections of *J. californica* were more attractive to female *X. saxeseni* than the older branch sections in round 2 of assay 6. The latter might be expected to exhibit a greater probability for fermentation and ethanol release.

*Hypothenemus eruditus* was trapped almost exclusively at the NCGR; however, two specimens were trapped at the ATRC in September 2018 and April 2019, respectively, during seasonal flight monitoring. There are no previous records of this beetle from Butte County, and this is the northernmost record of this invasive species in California (Seybold et al., 2016). Early in its invasion of California, it was found primarily along the southern Pacific Coast (Bright & Stark, 1973) and then later in the Central Valley (Seybold et al., 2016). It has a broad host range (e.g., Bright, 2014). From a separate study at the ATRC, we trapped two other specimens of *H. eruditus* during summer 2018, confirming its presence at this site. Response data from assays 5 and 6 at the NCGR suggest that female *H. eruditus* are active in flight in the spring and in the fall, and they also likely detect *J. californica* through olfaction while in flight. As was the case with female *X. saxeseni*, new branch sections of *J. californica* were more attractive to female *H. eruditus* than the older branch sections in round 2 of assay 6.

Branch sections of *J. major* also elicited higher landing rates by male *N. brevipennis* than branch sections of *J. californica*. A lack of a significant response for one host over the other by females may be attributable to the relatively low number of females caught in the study. Assays 1, 3, 4, and 6 showed a significant difference in male vs. female *N. brevipennis* catches with sex ratios ranging from 5:1 to 39:1 in favor of the males. This invasive beetle is native to Europe. In California it has been detected in *Juglans*, *Quercus*, and *Ficus* (Linsley, 1963). Not much is known biologically of this species, so it is difficult to explain the large difference in male and female catches in the landing assays. Seasonal trap catches at the ATRC showed a more even distribution of both sexes, but this may have been a consequence of random capture in traps baited with the *P. juglandis* pheromone. Male *N. brevipennis* may precede females phenologically to await mating opportunities. Although it is unknown where mating takes place, we presume that oviposition occurs on moribund twigs of *J. regia* where we have observed many larvae and adult emergence holes (Lona, 2019). Because of its strong association with Eurasian *J. regia*, it is not surprising that the landing response of *N. brevipennis* to *J. californica* was relatively weak. We did not detect this species during assay 5, because our seasonal survey trapping and all other landing assay trap catches suggest that the peak flight period is April or May to June, and earlier than the California flight peak (June–August) described by Linsley (1963). *Gildoria* sp., the potential parasitoid of *N. brevipennis*, is likely an undescribed species (R Zuparko & Y Braet, pers. comm.). Flight of *Gildoria* was largely contemporaneous with the flight of *N. brevipennis* (assays 3, 4, and 6). However, few *Gildoria* were trapped during assay 5 at the NCGR (i.e., fall of 2016). Rearing records (SJ Seybold, unpubl.) suggest a major emergence of *Gildoria* in March through June from small-diameter twigs of *J. regia*. For future studies, assays targeting *N. brevipennis* or *Gildoria* should be conducted during their periods of peak flight (April/May to June).

Subcortical insect species from eight families were trapped in the two northern California walnut orchards. Fewer Scolytidae were present at the ATRC. Surprisingly, one species, *X. affinis*, thought native to eastern North America, as well as Central and South America (Rabaglia et al., 2006; Bright, 2014), was trapped in 2015 in a seasonal survey trap at the NCGR and in 2016 while attempting to land on *J. major* at the ATRC. This species has not been reported previously from western North America. The presence of *X. affinis* at the ATRC was confirmed by the collection of a second specimen during an unrelated study conducted in the fall of 2018. A putative vector of *G. morbida* in the eastern USA, *Stenomimus* sp. (Curculionidae), was trapped only at the NCGR during assays 5 and 6. The extremely low rate of landing by this taxon in our assays suggests that it will likely not play a significant role in the transmission of *G. morbida* in California. A number of other herbivorous insects, parasitoids, and predaceous insects were present in both locations and most assays.

Our research may serve as a guide for designing and scheduling future studies at the NCGR and the ATRC, as well as similar sites throughout California. To further study the susceptibility of *J. californica* and *J. major* to *P. juglandis*, the next step should be to allow *P. juglandis* to land on cut branches of the two hosts and investigate their reproductive success rate. As our study found indications of long-range olfactory cues, examining the volatiles including the amount of ethanol emitted from the two host plants may provide further insight on the host selection by *P. juglandis* and associated beetles such as *X. saxeseni*, *H. eruditus*, and *N. brevipennis*.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Accession numbers of branches collected from *Juglans californica* and *J. major* trees at locations NCGR (USDA ARS National Clonal Germplasm Repository) and UCD (University of California at Davis walnut collection) for assays 1-5 of landing rate of *Pityophthorus juglandis*.

**Table S2.** Branches of *Juglans californica* collected from multiple locations in the native range in Southern California for assay 6 of landing rate of *Pityophthorus juglandis*.