Behavior

OXFORD

Flight Capacity of the Walnut Twig Beetle (Coleoptera: Scolytidae) on a Laboratory Flight Mill

Aubree M. Kees,[1,2] Andrea R. Hefty,[1,3] Robert C. Venette,[4] Steven J. Seybold,[5] and Brian H. Aukema[1]

[1]Department of Entomology, University of Minnesota, 1980 Folwell Ave., 432 Hodson Hall, St. Paul, MN 55108 (wilke137@umn.edu; ahefty@fs.fed.us; bhaukema@umn.edu), [2]Corresponding author, e-mail: wilke137@umn.edu, [3]US Department of Agriculture Forest Service, Forest Health Protection, Region 5, 602 S. Tippecanoe Ave., San Bernardino, CA 92408, [4]USDA Forest Service Northern Research Station, 1561 Lindig St., St. Paul, MN 55108 (rvenette@fs.fed.us), and [5]USDA Forest Service Pacific Southwest Research Station, HDH001 (F039) Orchard Park Drive, Rm 116, Davis, CA 95616 (sjseybold@gmail.com)
Subject Editor: Jeremy Allison

Abstract

The walnut twig beetle, *Pityophthorus juglandis* Blackman, and associated fungus *Geosmithia morbida* Kolařík, Freeland, Utley, & Tisserat constitute the insect–fungal complex that causes thousand cankers disease in walnut, *Juglans* spp., and wingnut, *Pterocarya* spp. Thousand cankers disease is responsible for the decline of *Juglans* species throughout the western United States and more recently, the eastern United States and northern Italy. We examined the flight capacity of *P. juglandis* over 24-h trials on a flight mill in the laboratory. The maximum total flight distance observed was ~3.6 km in 24 h; however, the mean and median distances flown by beetles that initiated flight were ~372 m and ~158 m, respectively. Beetles flew for 34 min on average within a 24-h flight trial. Male and female flight capacities were similar, even though males were larger than females (0.64 vs. 0.57 mm pronotal width). Age postemergence had no effect on flight distance, flight time, or mean flight velocity. The propensity to fly, however, decreased with age. We integrated results of flight distance with propensity to fly as beetles aged in a Monte Carlo simulation to estimate the maximum dispersal capacity over 5 d, assuming no mortality. Only 1% of the insects would be expected to fly >2 km, whereas one-third of the insects were estimated to fly <100 m. These results suggest that nascent establishments remain relatively localized without anthropogenic transport or wind-aided dispersal, which has implications for management and sampling of this hardwood pest.

Key words: dispersal, thousand cankers disease, *Geosmithia morbida*, *Pityophthorus juglandis*, sexual dimorphism

The walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae, *sensu* Bright 2014), is a phloeophagous beetle that is native to the southwestern United States and Mexico (Blackman 1928, Bright and Stark 1973, Bright 1981, Rugman-Jones et al. 2015). This beetle and an associated fungus, *Geosmithia morbida* M. Kolařík, E. Freeland, C. Utley & N. Tisserat et al. (2011), constitute the insect–fungal complex that causes thousand cankers disease (TCD; Tisserat et al. 2009, Kolařík et al. 2011, Seybold et al. 2013b). Several native and introduced walnuts, *Juglans* spp., and wingnuts, *Pterocarya* spp., are hosts for *P. juglandis* and *G. morbida* in the United States (Newton and Fowler 2009, Flint et al. 2010, Zerillo et al. 2014, Rugman-Jones et al. 2015, Hishinuma et al. 2016, Hefty 2016). Susceptibility to *G. morbida* varies among these hosts (Flint et al. 2010, Utley et al. 2013). Eastern black walnut, *Juglans nigra* L., is one of the most susceptible species to fungal infection (Tisserat et al. 2009, 2011; Utley et al. 2013) and beetle colonization (Hefty 2016).

Host selection by bark beetles such as *P. juglandis* is mediated by various host characteristics and physiological cues that may elicit host acceptance by the pioneering sex. Once a suitable host is selected by a male, in the case of *P. juglandis*, boring will occur into the outer bark and phloem. The male will initiate gallery construction and will be joined by at least two females in the nuptial chamber (Kirkendall 1983). After mating has occurred, females will tunnel through the phloem creating an egg gallery, laying eggs along the wall of this gallery. Emerged larvae then tunnel perpendicularly to the egg gallery and form a network of larval mines through the phloem. It is this intrusive feeding and subsequent canker development around the beetle galleries caused by *G. morbida* that causes TCD (Kolařík et al. 2011). As the name suggests, the development of a large number of cankers leads to the decline and mortality of the host.

Widespread ornamental plantings of eastern black walnut, *J. nigra*, and English walnut, *Juglands regia* L., may have facilitated geographic range expansion of *P. juglandis* in the western United States (Graves et al. 2009, Tisserat et al. 2011). In recent years, *P. juglandis* has also been detected in the eastern states of Tennessee,

Virginia, Pennsylvania, North Carolina, Maryland, Ohio, and Indiana, threatening native populations of *J. nigra* in those regions (Newton and Fowler 2009; Cranshaw 2011; Seybold et al. 2012a, 2016; Utley et al. 2013; Wiggins et al. 2014; Rugman-Jones et al. 2015). In 2013 and 2014, *P. juglandis* and *G. morbida* were found in *J. nigra* and *J. regia* in northern Italy, the first record of these agents in Europe, generating international concern (Montecchio and Faccoli 2014, Montecchio et al. 2014, Faccoli et al. 2016). The European distribution now includes four regions across northern Italy (Montecchio et al. 2016).

Recent phylogeographic analyses of >60 American populations of *P. juglandis* provide evidence that its range expansions are due, in part, to anthropogenic movement of infested wood (Rugman-Jones et al. 2015). Walnut is highly prized by woodworkers for crafting furniture, gunstocks, guitars, and other items (Newton and Fowler 2009, Tisserat et al. 2009). *Juglans nigra*, in particular, is one of the most highly valued timber species in North America. Moreover, walnut wood is utilized as firewood and considered good for home heating (Newton and Fowler 2009). Anthropogenic spread via wood or wood products is not unique to *P. juglandis*, as the movement of other invasive forest insects such as the emerald ash borer, *Agrilus planipennis* Fairmaire, provides strong evidence that the movement of firewood is a high-risk pathway for dispersal of bark and wood-boring beetles (Jacobi et al. 2012, Dodds et al. 2017).

Although anthropogenic movement of infested black walnut wood has facilitated the establishment of *P. juglandis* and thus TCD in the eastern United States, the additional contribution of natural dispersal to the insect's expansion across the western United States remains unknown (Cranshaw 2011). Little is known about the flight capacity of *P. juglandis*, for example. Determining the insect's flight characteristics in its natural environment is extremely challenging due to the insect's minute size (ca. 1.5–2.0 mm in length) and primarily endophytic life history (Seybold et al. 2013b, 2016).

Various techniques have been used to study the dispersal and movement of insects in the field and laboratory, ranging from harmonic radar (Machial et al. 2012) to mark–release–recapture techniques using tags (Gary 1970), etchings (Klingenberg et al. 2010), radioactive-isotope markers (Godwin et al. 1957), or other nondetrimental labels (Hagler and Jackson 2001). Among bark beetles, study techniques have included mark–release–recapture experiments on insects marked with paint or fluorescent powders prior to release in trap arrays (Dodds and Ross 2002, Costa et al. 2013); sampling experiments that net insects from airplanes (e.g., Jackson et al. 2008); post hoc statistical modeling of landscape damage patterns (de la Giroday et al. 2012); and flight mills (Atkins et al. 1966, Kinn 1986, Chen et al. 2010, Evenden et al. 2014, Fahrner et al. 2014, Lopez et al. 2014). Flight chambers and tethered flight apparatuses can measure flight characteristics that are difficult to capture in the insect's natural environment (Stinner et al. 1983, Taylor et al. 2010).

Here, we investigate the flight capacity of *P. juglandis* with a computer-monitored flight mill in the laboratory. Previous studies have examined diurnal flight patterns of *P. juglandis* in response to aggregation pheromone-baited traps in the field (Seybold et al. 2012b, Chen and Seybold 2014), but knowledge of the basic bioenergetic capabilities of this beetle remains limited. Our objectives were to assess the effect of sex and age on flight characteristics of this insect, including distance flown, flight velocity, and total flight time. Our goal was to characterize the flight potential of *P. juglandis* to improve our understanding of the natural dispersal of this hardwood pest and thus inform assessments of risk posed by the future range expansion of this insect.

Materials and Methods

Insects
All *P. juglandis* were obtained from infested branch sections from hybrid black walnut, *Juglans hindsii* × (*J. nigra* × *J. hindsii/Juglans californica*), collected in Sutter County, CA (39° 03.681′ N, 121° 36.818' W, 19.2 m elevation). The branch sections were ∼5 cm in diameter and 23 cm long, and shipped in February 2014 and March 2014, by overnight courier to the MAES/MDA biosafety level 2 Insect Biocontrol Facility at the University of Minnesota, St. Paul, where flight assays were conducted. Shipment and handling were conducted under the terms and conditions specified in Permits P526P-12-01650 and P526P-12-02498 from the US Department of Agriculture, Animal and Plant Health Inspection Service. Upon receipt, the branch sections were stored at constant room temperature (21 °C) and humidity (50% RH) in 3.8-liter plastic containers (ULINE, Pleasant Prairie, WI). *Pityophthorus juglandis* were allowed to emerge from cut branch sections, and collected and used for flight within 24 h, unless the experimental protocol required otherwise. When preparing a flight mill experiment, the beetles were held briefly in Petri dishes (140 by 20 mm) with moistened Kimwipes (Kimberly-Clark, Irving, TX) prior to attachment to the tether arm of the flight mill.

Flight Mill
Twelve computer-monitored flights mills, described by Fahrner et al. (2014), were used to investigate the flight capacity of *P. juglandis*. The tether arm of the flight apparatus was constructed from solid 33 American wire gauge (diameter: ∼0.171 mm) copper wire, to form a 5.5-cm tether arm to which the insect was attached. General attachment procedures are described by Fahrner et al. (2014) and are only summarized briefly here. For attachment to the tether arm, insects were gently retrieved from Petri dishes with a fine tip paintbrush and placed onto an icepack to slow their activity and limit movement. Once an insect was sufficiently chilled, the tip of the copper tether arm was dipped in a droplet of cyanoacrylate glue (Loctite Super Glue Gel; Henkel Corporation, Westlake, OH) and lightly pressed against the surface of the pronotum. Care was taken to ensure full movement of the elytra so as to not inhibit wing movement for flight. This glue was effective at securing the insects, and previous laboratory studies found no evidence of toxic effects on Warren root collar weevils, *Hylobius warreni* Wood, over the course of up to one year (Machial et al. 2012). The terminal 5 mm of the copper wire was then bent 90°, so the insect was facing perpendicularly to the tether arm, resulting in a radius of 5 cm for the final tether arm (Fahrner et al. 2014). Due to the small size of the insect, a counterweight was not required to balance the tether arm. Once the insect was successfully attached, the tether arm was placed on the flight mill to begin recording flight. Flight initiation was spontaneous and not instigated by manipulation of any kind.

Defining a Flight
An infra-red (IR) sensor recorded all sensitive movements of the tether arm as raw phase change data, including rare but spurious movements due to air currents or accidental bumps during assay initiation; any of which may have been identified as unrealistic flight speeds. Therefore, a limit for maximum speed was set at 0.45 m/s (1.62 km/h), based on timed observations of *P. juglandis* flight during preliminary assays, to exclude any potential misrecordings (Fahrner et al. 2014). Moreover, bouts of flight were only included in analyses if a minimum threshold of three full revolutions was met.

Experimental Protocols

Beetles were obtained from several host branch sections and randomly chosen for flight experiments. All flight trials occurred at room temperature (21.5 °C ± 0.04) and a low relative humidity (18.9% ± 0.43; mean ± SE) over 24 h in constant light (2700K fluorescent; ~1955 lux). Insects were not re-used following a 24-h flight trial. A maximum of 12 beetles was flown on any given day. The mortality of each beetle postflight was determined using leg movement as an indicator of survival. Survivors were placed in a Petri dish (94 by 16 mm) with a moistened Kimwipe and monitored daily for mortality. Once dead, all insects were stored at −80 °C in 0.5-ml microcentrifuge tubes. A subset of 60 insects was chosen at random for pronotal width measurements. Measurements were conducted at 40× magnification with a Leica MZ6 microscope (Wetzlar, Germany) connected to a real-time camera and digital micrometer.

Two separate flight experiments were conducted to test the effects of sex (i.e., male or female) and age (i.e., number of days postemergence) on the flight ability of *P. juglandis*. To determine the effect of sex on flight capacity, newly emerged beetles were selected randomly and assigned to channels of the flight mill on a given day. Only active (i.e., walking) beetles were chosen. The sex of each specimen was determined, prior to flight, under a microscope by the presence of a dense brush of setae on the female frons and the presence of granules on the male elytral declivity (Bright 1981, Seybold et al. 2013a). A total of 282 insects were placed on the flight mill during this experiment.

During the second flight experiment, which characterized how flight capacity changes with age postemergence, insects of varying ages were assigned randomly to channels of the flight mill on a given day. Beetles between 1 and 5 d postemergence were tested. Newly emerged beetles were collected and held in Petri dishes with moistened Kimwipes at room temperature (21.5 °C ± 0.04) for up to 5 d. Active insects were selected randomly from each age group, and used once in a flight trial. In addition, the sex of each beetle was recorded to account for any confounding effect of sex on flight. In this flight trial, a total of 372 insects were placed on the flight mill, with between 67–84 beetles in each age cohort.

Flight Mill Data

Extraction of flight metrics from the raw phase change data was conducted with R (R Development Core Team 2014) as described by Fahrner et al. (2014). The flight metrics included total flight time, distance, and velocity for each individual placed on the flight mill. Beetles often fly multiple times instead of one continuous flight; therefore, the total flight distance and total flight time of all bouts of flight for an individual were used for analysis, subject to the constraints defined earlier. Mean flight velocity was calculated as the total flight distance divided by the total active flight time for an individual.

Statistical Analyses

All data were analyzed by using R (R Development Core Team 2014). Linear models were used to analyze the relationships between the response variables such as distance flown, flight velocity, total flight time, and pronotal width with explanatory variables such as sex and age. For the first experiment investigating the effect of sex on flight, sex was fit as a factor in an ANOVA. Regression was used for the second experiment investigating the effect of age on flight. Graphs of the residual plots were examined for all models to check assumptions of normality and homoscedasticity of the errors.

Variance-stabilizing logarithmic transformations were used to transform distance, velocity, and time data. Logistic regressions were used to analyze the propensity of flight with sex and age postemergence.

Monte Carlo Simulation

A Monte Carlo simulation was used to integrate flight distance with age and estimate flight capacity of a population of beetles over 5 d. The simulation draws a random sample from a binomial distribution parameterized with day-specific empirical data of the likelihood of an individual chosen at random to fly on a given day if placed on the flight mill (i.e., individuals were less likely to attempt flight as they aged; see Results). This random variable results in a flight or nonflight on a given day, i. If this draw results in a flight, a distance value, X_i, is selected from the empirical distribution of flight data on day i; if a nonflight was drawn, X_i is equal to zero. These steps are repeated for $i = 1...5$ d and the flight distances are summed for a total flight distance over 5 d (total flight = $\sum_{i=1}^{5} X_i$) to obtain the expected flight distance of an individual insect in the 5 d postemergence. These steps were repeated 100,000 times to estimate the maximum dispersal capacity of a population of beetles emerging from a host under the assumptions that dispersal was perfectly linear and no mortality occurred.

Results

A total of 654 *P. juglandis* were placed on the flight mills during these two experiments. In total, 45% (or 293 of these insects) initiated flight. The maximum total flight distance was ~3.6 km in 24 h of tethered flight. Of beetles that initiated flight, the mean and median distance flown were ~372 m and 158 m, respectively. The longest distance flown in a single bout of flight was 1.2 km. The highest velocity reached in a single bout of flight was 0.448 m/s. Beetles flew a mean total of 34 min within 24 h. Ninety-three percent of beetles that initiated flight completed their final flight in the first 10 h (Fig. 1). The distribution of last flight recordings was highly skewed, however, such that the mean hour of the last flight event occurred 3 h 58 min into the trial. Only three of the 293 beetles that flew were still flying between 20–24 h post trial-initiation (Fig. 1). Only nine of the 654 beetles placed on the mill were found to be alive when the trial ended.

In determining the effect of sex on flight capacity, a total of 178 *P. juglandis* flew when placed on the flight mill (95 females, 83 males) from an initial cohort of 282 insects (126 females, 156 males). The proportion of beetles that initiated flight versus those that did not fly did not vary by sex (Z = 0.86, P = 0.39). Female
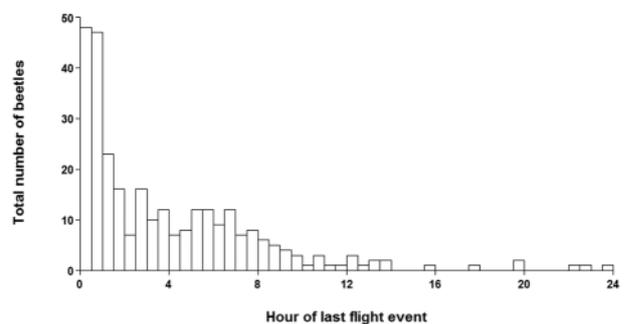

Fig. 1. Hour of last flight event of male and female walnut twig beetles, *P. juglandis*, recorded on a computer-monitored flight mill over a 24-h period (*n* = 293 beetles).

P. juglandis flew a mean (±SE) distance of 331.9 ± 46.5 m, whereas males flew 495.0 ± 68.8 m ($F_{1, 176} = 2.80$, $P = 0.10$) in a 24-h period (Fig. 2A). Mean flight velocity was similar between sexes (0.172 ± 0.0039 m/s for females, 0.177 ± 0.005 m/s for males) over
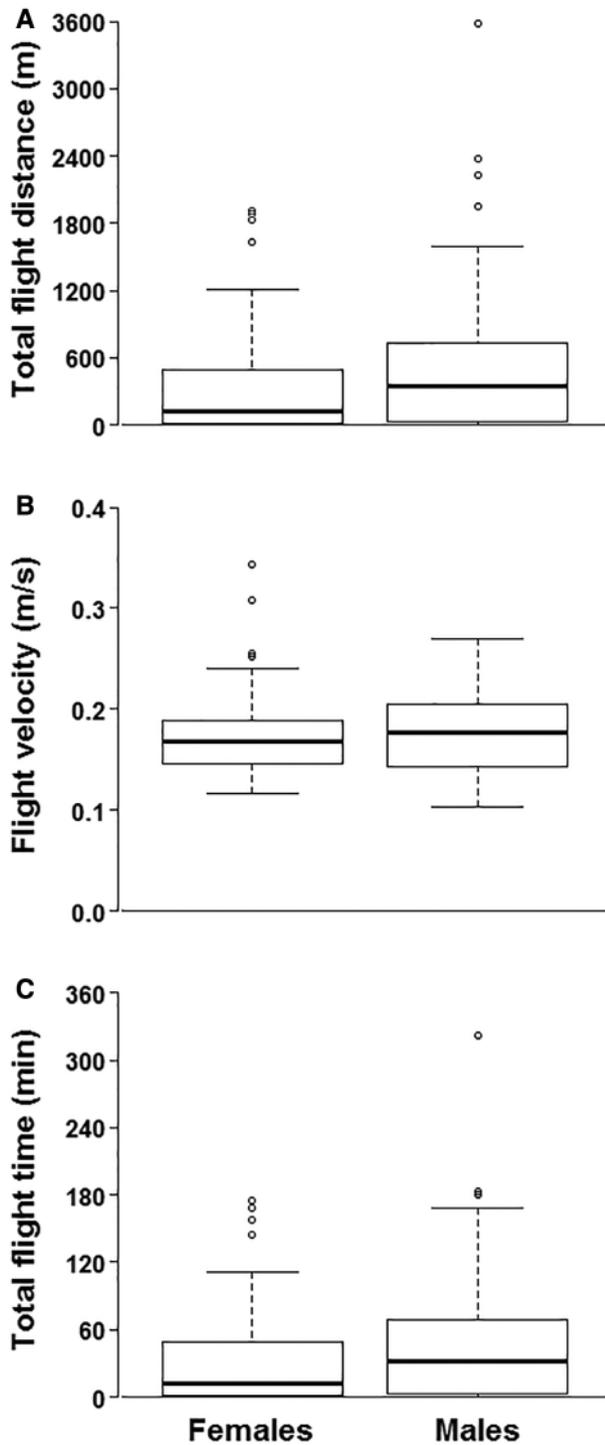
a 24-h flight trial ($F_{1, 176} = 0.51$, $P = 0.48$; Fig. 2B). Males spent 52 percent more time in flight, on average, than females, although this difference was not statistically significant ($F_{1, 176} = 2.62$, $P = 0.11$; Fig. 2C). Male mean pronotal width (0.64 ± 0.01 mm) was significantly larger than female mean pronotal width (0.57 ± 0.01 mm; $F_{1, 58} = 25.72$, $P < 0.001$; $n = 30$ for each sex; Fig. 3).

In determining the effect of age following emergence from the host on flight capacity, a total of 372 *P. juglandis*, ages 1 to 5 d postemergence, were placed on the flight mill, of which 115 flew. The propensity to fly decreased with age ($Z = -7.38$, $P < 0.001$; Fig. 4). Between 1 and 5 d following emergence from the host, the likelihood that a beetle would initiate flight decreased from 60
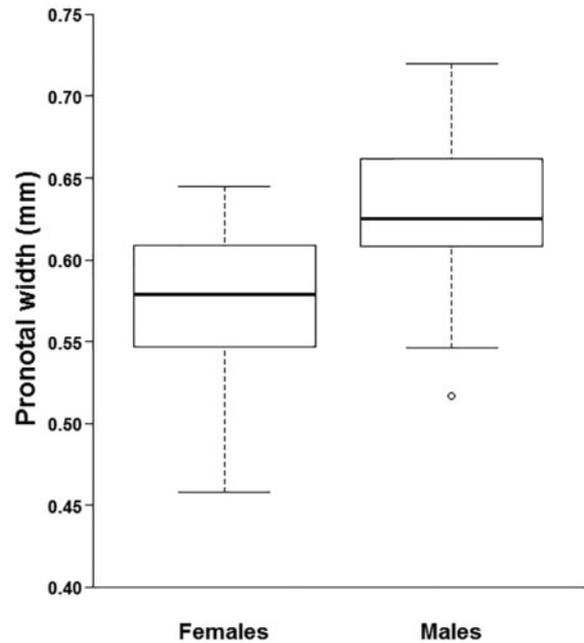

Fig. 2. Comparison of flight metrics of female and male walnut twig beetles, *P. juglandis*, on a computer-monitored flight mill over a 24-h period ($n = 95$ females and 83 males). (**A**) Total flight distance. (**B**) Flight velocity. (**C**) Total flight time. Boxplots represent quartiles and median; whiskers represent either the maximum and minimum observed values or 1.5 times the interquartile range of the data (whichever is smaller); and dots represent extreme values.

Fig. 3. Comparison of pronotal widths of female and male walnut twig beetles, *P. juglandis* ($n = 30$ females and 30 males; $F_{1, 58} = 25.72$, $P < 0.001$). Boxplots are as described in Figure 2.
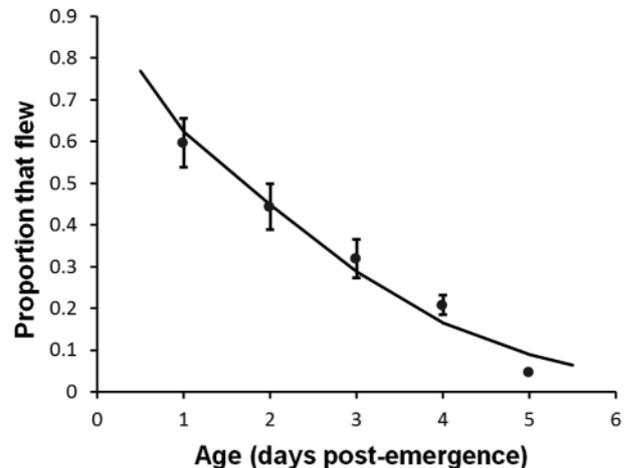

Fig. 4. Effect of age on the proportion (±SE) of walnut twig beetles, *P. juglandis*, which initiated flight 1 to 5 d postemergence on a computer-monitored flight mill. Probability of flight equals $e^y/(1 + e^y)$ where $y = 1.20689 - 0.70562$ (age in days postemergence); $n = 67, 72, 72, 77,$ and 84 insects from days 1 to 5.

percent to less than one percent. However, total flight distance ($F_{1, 113} = 2.27$, $P = 0.13$), flight velocity ($F_{1, 113} = 2.247$, $P = 0.14$), and total flight time did not change with age among those individuals that flew ($F_{1, 113} = 1.46$, $P = 0.23$; Fig. 5).

Results from a Monte Carlo simulation integrating propensity to fly with beetle age indicated that *P. juglandis* adults could potentially fly a mean distance of 491.9 m over 5 d (median distance = 280 m). Approximately one-third of insects would be expected to fly <100 m, whereas 1% of the insects would fly >2 km in a 5-d period (Fig. 6).

Discussion

Our findings are consistent with a hypothesis that natural dispersal may only contribute marginally to the incremental spread of this hardwood pest. Given the tiny size of *P. juglandis*, we expect that natural flight capacity is limited to not more than 3 or 4 km at the extremes (Fig. 6) and that insects will remain somewhat localized at sites of introduction in the short term. These insects may reduce flight activity in the presence of a light breeze, for example; field studies have noted that fewer *P. juglandis* are captured when wind speeds exceed 4 km/h (Chen and Seybold 2014), which is 2.5× the maximum speed noted on our flight mill. Localized stationarity of new populations appears to be consistent with detection survey data for beetles in Butler Co., OH, and Bucks Co., PA (S.J.S., personal observations).

Patterns of spread of invasive species are often characterized by stratified dispersal (e.g., Hengeveld 1988, Liebhold and Tobin 2008), which reflects a combination of localized dispersal with long-distance movement perhaps aided by human transport (Liebhold et al. 1995, 2012) or wind (de la Giroday et al. 2011); both factors we cannot measure. Some species of bark beetles orient toward dark objects on a light background upon emergence, such that a small proportion of the population rises skyward and above the forest canopy, where they become entrained in air currents (Chapman 1962, Shepherd 1966, Safranyik et al. 1989). Within meso-scale atmospheric currents, insects drift as inert particles with variable amounts of flight control, a process known as aeolian dispersal (Lewis and Dibley 1970, Taylor 1974, de la Giroday et al. 2011). Wind-aided dispersal can profoundly impact small-bodied insects such as bark beetles (Furniss and Furniss 1972, Nilssen 1984, Crossley and Hogg 2015). *Ips typographus*, for example, can reach distances of >50 km (Botterweg 1982, Forsse and Solbreck 1985), and *Dendroctonus ponderosae* Hopkins has been observed to travel hundreds of kilometers in a single day (Furniss and Furniss 1972, Safranyik and Carroll 2006, Jackson et al. 2008, de la Giroday et al. 2011).
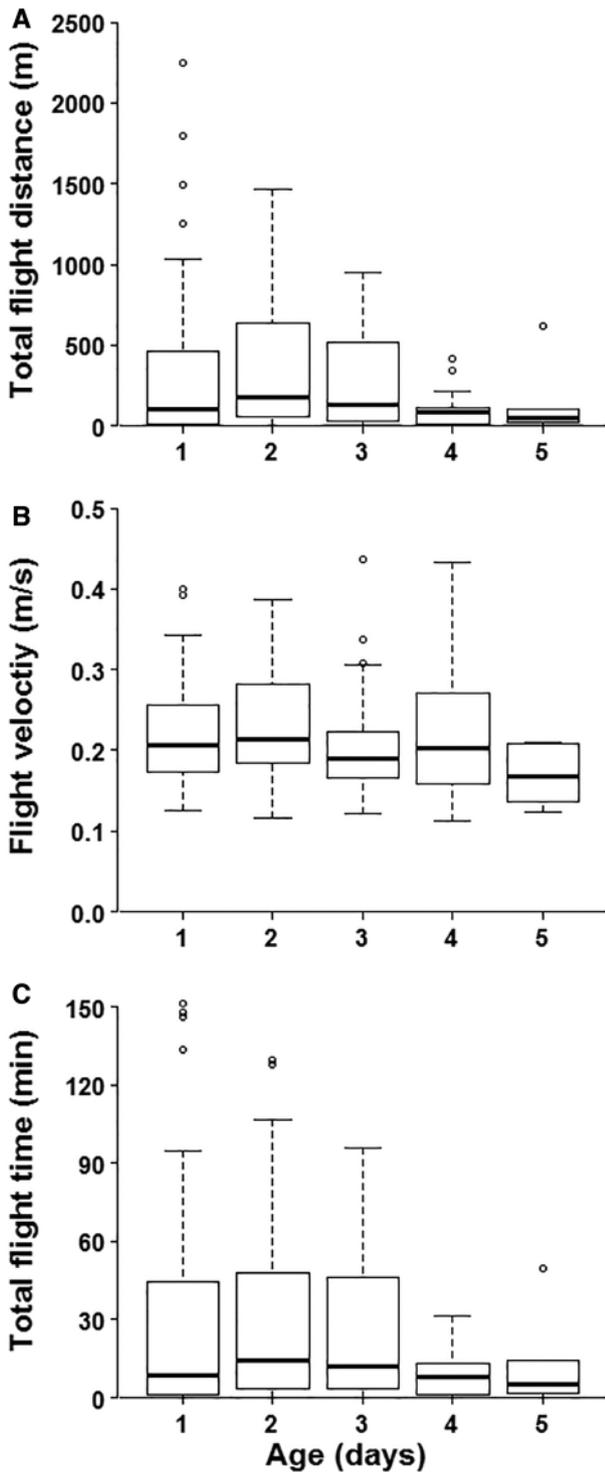

Fig. 5. Comparison of flight metrics of walnut twig beetles, *P. juglandis*, 1 to 5 d postemergence on a computer-monitored flight mill. $n = 40$, 32, 23, 14, and 6 fliers for days 1 to 5. (**A**) Total flight distance. (**B**) Flight velocity. (**C**) Total flight time. Boxplots are as described in Figure 2.
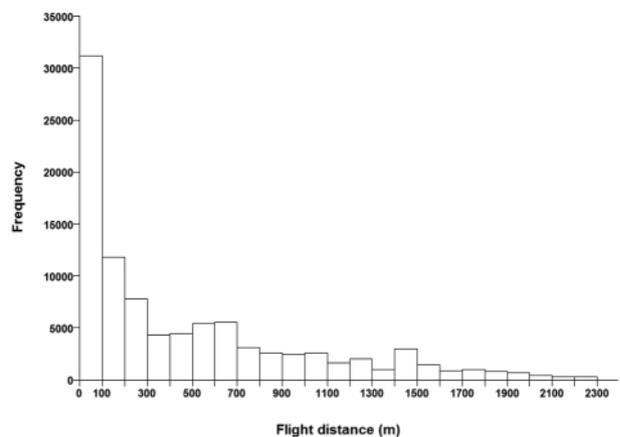

Fig. 6. Histogram of potential total flight distance over 5 d postemergence by walnut twig beetles, *P. juglandis*. A Monte Carlo simulation integrates empirical results from experiments reflecting propensity to fly and distance flown at different ages postemergence. Histogram reflects a hypothetical distribution of 100,000 insects.

Long-distance dispersal may be more critical to these aggressive species of bark beetles that exhaust host resources in as little as one generation (Raffa et al. 2005) vs. less-aggressive species that may complete multiple generations on the same host or encounter abundant hosts under a regional stress (Botterweg 1982, Raffa et al. 2008). In urban environments, *P. juglandis* seems to recolonize progressively lower sections of stems of natal host trees, and/or attacks stressed neighboring trees along streets or in poorly irrigated locations outside of their natural ecological habitats (Seybold et al. 2016). Nonetheless, reduced captures of *P. juglandis* when wind speeds exceed 4 km/h (Chen and Seybold 2014) may simply reflect wind-aided dispersion away from the trapping environment rather than lack of flight activity.

If newly established populations of *P. juglandis* do remain relatively localized upon introduction, this may present a serious challenge for detecting and sampling these unknown populations on the landscape (Venette et al. 2002). Many hundreds if not thousands of pheromone-baited funnel traps would need to be deployed to locate the hypothetical widely dispersed, but spatially constrained populations (Seybold et al. 2013a). If TCD symptoms on the host are used to locate newly established populations of *P. juglandis* (USDA FS PPQ 2016), then timely detection might be further complicated by the lag time between infestation and TCD symptom expression (Tisserat et al. 2009). Once located, however, if *P. juglandis* populations have a slow rate of spread, then, theoretically at least, large numbers of suitably spaced pheromone-baited funnel traps could be used at known sites of introduction to mass trap and reduce resident populations (Jakuš 1998, Schlyter et al. 2001, El-Sayed et al. 2006).

We did not find significant differences in flight characteristics between male and female bark beetles over the first few days of flight, similar to the findings of Evenden et al. (2014) for *D. ponderosae*. Among bark beetles, the pioneering sex is typically the larger sex when a sexual size dimorphism is present in adults (Foelker and Hofstetter 2014). Female insects may fly farther in some species of *Dendroctonus*, where females are the host-selecting sex (e.g., Kinn et al. 1994, Chen et al. 2011), perhaps because sustained flight is necessary for pioneering females to successfully locate suitable host trees (Evenden et al. 2014). The absence of sex-specific flight advantages in this system is consistent with other studies in which males are the pioneering sex (e.g., Forsse and Solbreck 1985), as is the case with *P. juglandis* (Seybold et al. 2016). For example, Botterweg (1982) observed no effect of sex, size, or fat content on the dispersal of the Eurasian spruce bark beetle, *Ips typographus* L., in the field. It is possible that sex-related differences in flight do exist among individual *P. juglandis*, but detection of these differences may only be possible after successive 24-h flight periods (Chen et al. 2011), as larger-bodied individuals have enhanced lipid reserves to power flight (Williams and Robertson 2008, Lease and Wolf 2011, Kaufmann et al. 2013).

We did not find a significant decrease in flight capacity with age, unlike previous laboratory studies of bark beetles (Chen et al. 2011, Evenden et al. 2014). Evenden et al. (2014), for example, noted a decline in the dispersal capacity of *D. ponderosae* with age postemergence, which they attributed to depletion of lipid stores during storage and starvation prior to placement on the flight mill (Gries et al. 1990, Evenden et al. 2014). Some species of bark and wood-boring insects are known to feed after emergence from the host tree, such as *A. planipennis*, which feeds on ash leaves for 2–3 wk prior to ovipositing (Jennings et al. 2014). Because *D. ponderosae* is not known to feed after emergence, Evenden et al. (2014) concluded

that starvation before flight was reflective of dispersal under field conditions. Similarly, in the absence of any data on the pre- or postemergence feeding behavior of new adult *P. juglandis* on its host, we cannot conclude that the experimental conditions of this study do not similarly reflect natural age effects on dispersal.

We do exercise caution when relating laboratory-based flight potential to natural dispersal, even though several studies have demonstrated good relationships between measurements of insects in tethered flight and field observations of dispersal (Forsse and Solbreck 1985, Jactel and Gaillard 1991, Evenden et al. 2014). Tethered flight studies in the laboratory limit the influence of many abiotic factors that affect flight during natural dispersal, such as ambient air temperature, light intensity, and barometric pressure. Each of these variables has been shown previously to be associated with *P. juglandis* flight (Chen and Seybold 2014). Relative humidity (RH) can also influence flight, especially for small-bodied insects (Zhang et al. 2008, Keppner and Jarau 2016). Fahrner et al. (2015), for example, found that the flight speed of *A. planipennis* declined with an increase in humidity during a 24-h trial on the same flight mills used herein. In another laboratory study, flight frequency of white pine cone beetle, *Conophthorus coniperda* (Schwarz), increased with exposure to dry air (Henson 1962). The ambient humidity levels for our study were relatively low, with daily means between 11–33% RH in the quarantine facility. The mortality of all but nine beetles by the end of the 24-h flight trial may have been due to desiccation caused by low humidity; however, we did not find an effect of humidity on distance flown ($F_{1, 291} = 0.14$, $P = 0.71$). In the field, increased humidity is associated with decreased flight of *P. juglandis* (Chen and Seybold 2014). This pattern may be due to a higher, more costly wing-beat frequency instigated by higher humidities (Bhan et al. 2014), and would be consistent with cessation of flight by the majority of individuals within 8 h (Fig. 1). Hypothetically, factors with biotic origins in the field such as the presence of host or nonhost volatiles or pheromone plumes may also impact dispersal of *P. juglandis*.

Populations of *P. juglandis* appear to have declined in recent years in the eastern United States, although recent detections in Italy (Montecchio et al. 2014, 2016) and characterizations of host ranges beyond *Juglans* spp. (Hishinuma et al. 2016, Hefty 2016) suggest the importance of monitoring this insect. As further information regarding the natural dispersal of *P. juglandis* becomes available, this basic knowledge of flight capability will inform management strategies to minimize the spread of this invasive pest.

Acknowledgments

References

Atkins, M. D. 1966. Laboratory studies on the behaviour of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. Can. Entomol. 98: 953–991. DOI 10.4039/Ent98953-9

Bhan, V., R. Parkash, and D. D. Aggarwal. 2014. Effects of body-size variation on flight-related traits in latitudinal populations of *Drosophila melanogaster*. J. Genet. 93: 103–112. DOI 10.1007/s12041-014-0344-5

Blackman, M. W. 1928. The genus *Pityophthorus* Eichh. in North America: a revisional study of the *Pityophthori*, with descriptions of two new genera and seventy-one new species, pp. 3–6. Technical Publication Bulletin NY State College of Forestry, Syracuse University, Syracuse, NY.

Botterweg, P. F. 1982. Dispersal and flight behaviour of the spruce bark beetle *Ips typographus* in relation to sex, size and fat content. J. Appl. Entomol. 94: 466–489.

Bright, D. E. 1981. Taxonomic monograph of the genus *Pityophthorus* Eichhoff in North and Central America. Mem. Entomol. Soc. Can. 118: 1–378.

Bright, D. E. 2014. A catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 3 (2000–2010), with notes on subfamily and tribal reclassifications. Insecta Mundi. 356: 1–336.

Bright, D. E., and R. W. Stark. 1973. The Bark and Ambrosia Beetles of California (Scolytidae and Platypodidae), p. 169. Bull. Calif. Insect Surv. University of California, Berkeley, CA.

Chapman, J. A. 1962. Field studies on attack flight and log selection by ambrosia beetle *Trypodendron lineatum* (Oliv.) (Coleoptera: Scolytidae). Can. Entomol. 94: 74–92.

Chen, H., Z. Li, and M. Tang. 2010. Laboratory evaluation of flight activity of *Dendroctonus armandi* (Coleoptera: Curculionidae: Scolytinae). Can. Entomol. 142: 378–387.

Chen, H., Z. Li, S.-H. Bu, and Z.-Q. Tian. 2011. Flight of the Chinese white pine beetle (Coleoptera: Scolytidae) in relation to sex, body weight and energy reserve. Bull. Entomol. Res. 101: 53–62.

Chen, Y., and S. J. Seybold. 2014. Crepuscular flight activity of an invasive insect governed by interacting abiotic factors. PLoS ONE 9: e105945.

Costa, A., A. Min, C. K. Boone, A. P. Kendrick, R. J. Murphy, W. C. Sharpee, K. F. Raffa, and J. D. Reeve. 2013. Dispersal and edge behaviour of bark beetles and predators inhabiting red pine plantations. Agric. For. Entomol. 15: 1–11.

Cranshaw, W. S. 2011. Recently recognized range extensions of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae), in the western United States. Coleopt. Bull. 65: 48–49.

Crossley, M. S., and D. B. Hogg. 2015. Potential overwintering locations of soybean aphid (Hemiptera: Aphididae) colonizing soybean in Ohio and Wisconsin. Environ. Entomol. 44: 210–222.

de la Giroday, H.-M.C.,. L. Carroll, B. S. Lindgren, and B. H. Aukema. 2011. Incoming! Association of landscape features with dispersing mountain pine beetle populations during a range expansion event in western Canada. Landsc. Ecol. 26: 1097–1110.

de la Giroday, H.-M. C., L. Carroll, and B. H. Aukema. 2012. Breach of the northern Rocky Mountain geoclimatic barrier: initiation of range expansion by the mountain pine beetle. J. Biogeogr. 39: 1112–1123.

Dodds, K. J., and D. W. Ross. 2002. Sampling range and range of attraction of *Dendroctonus pseudotsugae* pheromone-baited traps. Can. Entomol. 134: 343–355.

Dodds, K. J., P. Hanavan, and M. F. DiGirolomo. 2017. Firewood collected after a catastrophic wind event: the bark beetle (Scolytinae) and woodborer (Buprestidae, Cerambycidae) community present over a 3-year period. Agric. For. Entomol. Online. http://doi.wiley.com/10.1111/afe.12210.

El-Sayed, A. M., M. Suckling, C. H. Wearing, and J. A. Byers. 2006. Potential of mass trapping for long-term pest management and eradication of invasive species. J. Econ. Entomol. 99: 1550–1564.

Evenden, M. L., M. Whitehouse, and J. Sykes. 2014. Factors influencing flight capacity of the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae). Environ. Entomol. 43: 10.

Faccoli, M., M. Simonato, and D. Rassati. 2016. Life history and geographical distribution of the walnut twig beetle, *Pityophthorus juglandis* (Coleoptera: Scolytinae), in southern Europe. J. Appl. Entomol. 1–9. doi: 10.1111/jen.12299

Fahrner, S. J., P. Lelito, K. Blaedow, G. E. Heimpel, and B. H. Aukema. 2014. Factors affecting the flight capacity of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a classical biological control agent of *Agrilus planipennis* (Coleoptera: Buprestidae). Environ. Entomol. 43: 1603–1612.

Fahrner, S. J., P. Lelito, and B. H. Aukema. 2015. The influence of temperature on the flight capacity of emerald ash borer *Agrilus planipennis* and its parasitoid, *Tetrastichus planipennisi*: Implications to biological control. BioControl 60: 437–449.

Flint, M. L., D. Graves, and S. J. Seybold. 2010. Thousand cankers disease of walnuts spreads in California. CAPCA Advisor Magazine. 8: 36–39.

Foelker, C., and R. Hofstetter. 2014. Heritability, fecundity, and sexual size dimorphism in four species of bark beetles (Coleoptera: Curculionidae: Scolytinae). Ann. Entomol. Soc. Am. 107: 143–151.

Forsse, E., and C. Solbreck. 1985. Migration in the bark beetle *Ips typographus*: Duration, timing and height of flight. J. Appl. Entomol. 100: 47–57.

Furniss, M. M., and R. L. Furniss. 1972. Scolytids (Coleoptera) on snowfields above timberline in Oregon and Washington. Can. Entomol. 104: 1471–1478.

Gary, N. E. 1970. Magnetic retrieval of ferrous labels in a capture-recapture system for honey bees and other insects. J. Econ. Entomol. 64: 961–965.

Godwin, P. A., H. A. Jaynes, and J. M. Davis. 1957. The dispersion of radioactively tagged white-pine weevils in small plantations. J. Econ. Entomol. 50: 264–266.

Graves, A. D., T. W. Coleman, M. L. Flint, and S. J. Seybold. 2009. Walnut twig beetle and thousand cankers disease: Field identification guide. UC-IPM Website Publication, 2 pp., Nov. 21, 2009, (http://www.ipm.ucdavis.edu/PDF/MISC/thousand_cankers_field_guide.pdf)

Gries, G., W. W. Bowers, R. Gries, M. Noble, and J. H. Borden. 1990. Pheromone production by the pine engraver *Ips pini* following flight and starvation. J. Insect Physiol. 36: 819–824.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: current techniques and future prospects. Annu. Rev. Entomol. 46: 511–543.

Hefty, A. R. 2016. Risk of invasion by walnut twig beetle throughout eastern North America. PhD. dissertation, University of Minnesota, Minneapolis, MN.

Hengeveld, R. 1988. Mechanisms of Biological Invasions. J. Biogeogr. 15: 819–828.

Henson, W. R. 1962. Laboratory studies of the adult behavior of *Conophthorus coniperda* (Coleoptera: Scolytidae). III. Flight. Ann. Entomol. Soc. Am. 55: 524–530.

Hishinuma, S. M., P. L. Dallara, M. A. Yaghmour, M. M. Serillo, C. M. Parker, T. V. Roubtsova, T. L. Nguyen, N. A. Tisserat, R. M. Bostock, M. L. Flint, et al. 2016. Wingnut (Juglandaceae) as a new generic host for *Pityophthorus juglandis* (Coleoptera: Curculionidae) and the thousand cankers disease pathogen, *Geosmithia morbida* (Ascomycota: Hypocreales). Can. Entomol. 148: 83–91. DOI 10.4039/tce.2015.37.

Jackson, P. L., D. Straussfogel, B. S. Lindgren, S. Mitchell, and B. Murphy. 2008. Radar observation and aerial capture of mountain pine beetle, *Dendroctonus ponderosae* Hopk. (Coleoptera: Scolytidae) in flight above the forest canopy. Can. J. For. Res. 38: 2313–2327.

Jacobi, W. R., J. G. Hardin, B. A. Goodrich, and C. M. Cleaver. 2012. Retail firewood can transport live tree pests. J. Econ. Entomol. 105: 1645–1658.

Jactel, H., and J. Gaillard. 1991. A preliminary study of the dispersal potential of *Ips sexdentatus* (Boern) (Col., Scolytidae) with an automatically recording flight mill. J. Appl. Entomol. 112: 138–145.

Jakuš, R. 1998. A method for the protection of spruce stands against *Ips typographus* by the use of barriers of pheromone traps in north-eastern Slovakia. J. Pest Sci. 71: 152–158.

Jennings, D. E., P. B. Taylor, and J. J. Duan. 2014. The mating and oviposition behavior of the invasive emerald ash borer (*Agrilus planipennis*), with reference to the influence of host tree condition. J. Pest Sci. 87: 71–78.

Kaufmann, C., C. Reim, and W. U. Blanckenhorn. 2013. Size-dependent insect flight energetics at different sugar supplies. Biol. J. Linn. Soc. 108: 565–578.

Keppner, E. M., and S. Jarau. 2016. Influence of climatic factors on the flight activity of the stingless bee *Partamona orizabaensis* and its competition behavior at food sources. J. Comp. Physiol. A. 202: 691–699. DOI 10.1007/s00359-016-1112-1

Kinn, D. N. 1986. Studies on the flight capabilities of Dendroctonus frontalis and Ips calligraphus: Preliminary findings using tethered beetles. USDA Forest Service, Southern Forest Experiment Station, Research Note SO-324, p. 3.

Kinn, D. N., T. J. Perry, F. H. Guinn, B. L. Strom, and J. Woodring. 1994. Energy reserves of individual southern pine beetles (Coleoptera: Scolytidae) as determined by a modified phosphovanillin spectrophotometric method. J. Entomol. Sci. 29: 152–163.

Kirkendall, L. R. 1983. The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). Zool. J. Linn. Soc. 77: 293–352.

Klingenberg, M. D., N. Björklund, and B. H. Aukema. 2010. Seeing the forest through the trees: Differential dispersal of *Hylobius warreni* Wood within modified forest habitats. Environ. Entomol. 39: 898–906.

Kolařík, M., E. Freeland, C. Utley, and N. Tisserat. 2011. *Geosmithia morbida* sp. nov., a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on *Juglans* in U.S.A. Mycologia 103: 325–332.

Lease, H. M., and B. O. Wolf. 2011. Lipid content of terrestrial arthropods in relation to body size, phylogeny, ontogeny and sex. Physiol. Entomol. 36: 29–38.

Lewis, T., and G. Dibley. 1970. Air movement near windbreaks and a hypothesis on the mechanism of the accumulation of airborne insects. Ann. Appl. Biol. 66: 477–484.

Liebhold, A. M., W. L. MacDonald, D. Bergdahl, and V. C. Mastro. 1995. Invasion by exotic forest pests: A threat to forest ecosystems. For. Sci. 41: 50.

Liebhold, A. M., and P. C. Tobin. 2008. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53: 387–408.

Liebhold, A. M., E. G. Brockerhoff, L. J. Garrett, J. L. Parke, and K. O. Britton. 2012. Live plant imports: The major pathway for forest insect and pathogen invasions of the US. Front Ecol. Environ. 10: 135–143.

Lopez, V. M., M. N. McClanahan, L. Graham, and M. S. Hoddle. 2014. Assessing the flight capabilities of the goldspotted oak borer, *Agrilus auroguttatus* (Coleoptera: Buprestidae), with computerized flight mills. J. Econ. Entomol. 107: 1127–1135. http://dx.doi.org/10.1603/EC13525

Machial, L. A., B. S. Lindgren, R. W. Steenweg, and B. H. Aukema. 2012. Dispersal of Warren root collar weevils (Coleoptera: Curculionidae) in three types of habitat. Environ. Entomol. 41: 578–586.

Montecchio, L., and M. Faccoli. 2014. First record of thousand cankers disease *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans nigra* in Europe. Plant Dis. 98: 696.

Montecchio, L., G. Fanchin, M. Simonato, and M. Faccoli. 2014. First record of thousand cankers disease fungal pathogen *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans regia* in Europe. Plant Dis. 98: 1445. http://dx.doi.org/10.1094/PDIS-07-14-0719-PDN

Montecchio, L., M. Vettorazzo, and M. Faccoli. 2016. Thousand cankers disease in Europe: An overview. EPPO Bull. 46: 335–340.

Newton, L., and G. Fowler. 2009. Pathway assessment: Geosmithia sp. and Pityophthorus juglandis Blackman movement from the western into the eastern United States, U.S. Dept. of Ag., Animal and Plant Health Inspection Service, Washington, DC. (https://tn.gov/agriculture/publications/regulatory/tc_pathwayanalysis.pdf)

Nilssen, A. 1984. Long-range aerial dispersal of bark beetles and bark weevils (Coleoptera, Scolytidae and Curculionidae) in northern Finland. Ann. Entomol. Fenn. 50: 37–42.

R Development Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raffa, K. F., B. H. Aukema, N. Erbilgin, K. D. Klepzig, and K. F. Wallin. 2005. Interactions among conifer terpenoids and bark beetles across multiple levels of scale: An attempt to understand links between population patterns and physiological processes. Recent Adv. Phytochem. 39: 79–118.

Raffa, K. F., B. H. Aukema, B. J. Bentz, A. L. Carroll, J. A. Hicke, M. G. Turner, and W. H. Romme. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of bark beetle eruptions. BioScience 58: 501–517.

Rugman-Jones, P. F., S. J. Seybold, A. D. Graves, and R. Stouthamer. 2015. Phylogeography of the walnut twig beetle, *Pityophthorus juglandis*, the vector of thousand cankers disease in North American walnut trees. PLoS ONE 10: e0118264.

Safranyik, L., and A. L. Carroll. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. pp. 3–66. *In* L. Safranyik and B. Wilson (eds.), The mountain pine beetle: A synthesis of biology, management and impacts on lodgepole pine. Pacific Forestry Centre, Victoria, Canada.

Safranyik, L., R. Silversides, L. H. McMullen, and D. A. Linton. 1989. An empirical approach to modeling the local dispersal of the mountain pine beetle (*Dendroctonus ponderosae* Hopk.) (Col., Scolytidae) in relation to sources of attraction, wind direction, and speed. J. Appl. Entomol. 108: 498–511.

Schlyter, F., Q.-H. Zhang, G.-T. Liu, and L.-Z. Ji. 2001. A successful case of pheromone mass trapping of the bark beetle *Ips duplicatus* in a forest island, analyzed by 20-year time-series data. Integrated Pest Manag. Rev. 6: 185–196.

Seybold, S. J., T. W. Coleman, P. L. Dallara, N. L. Dart, A. D. Graves, L. Pederson, and S.-E. Spichiger. 2012a. Recent collecting reveals new state records and the extremes in the distribution of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in the United States. Pan-Pac. Entomol. 88: 377–380.

Seybold, S. J., J. A. King, D. R. Harris, L. J. Nelson, S. M. Hamud, and Y. Chen. 2012b. Diurnal flight response of the walnut twig beetle, *Pityophthorus juglandis*, to pheromone-bated traps in two northern California walnut habitats. Pan-Pac. Entomol. 88: 231–247.

Seybold, S. J., P. L. Dallara, S. M. Hishinuma, and M. L. Flint. 2013a. Detecting and identifying walnut twig beetle: monitoring guideline for the invasive vector of thousand cankers disease of walnut. UC IPM Program, University of California Agriculture and Natural Resources, p. 13.

Seybold, S. J., D. Haugen, J. O'Brien, and A. D. Graves. 2013b. Thousand cankers disease. USDA Forest Service, Northeastern Area State and Private Forestry Pest Alert, NA-PR-02-10, originally published May. 2010, reprinted Aug. 2010, Oct. 2011, and Feb. 2013, 2 pp. (http://www.na.fs.fed.us/pubs/detail.cfm?id=5225)

Seybold, S. J., R. L. Penrose, and A. D. Graves. 2016. Invasive bark and ambrosia beetles in California Mediterranean forest ecosystems. pp. 583–662, *In*: T. D. Paine and F. Lieutier (eds.). Insects and Diseases of Mediterranean Forest Systems, Springer International Publishing AG, Cham, Switzerland. DOI 10.1007/978-3-319-24744-1_21

Shepherd, R. F. 1966. Factors influencing the orientation and rates of activity of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). Can. Entomol. 97: 207–215.

Stinner, R., C. Barfield, J. Stimac, and L. Dohse. 1983. Dispersal and movement of insect pests. Annu. Rev. Entomol. 28: 319–335.

Taylor, L. R. 1974. Insect migration, flight periodicity and the boundary layer. J. Anim. Ecol. 43: 225–238.

Taylor, R.A.J., L. S. Bauer, T. M. Poland, and K. N. Windell. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a flight mill and in free flight. J. Insect. Behav. 23: 128–148. DOI 10.1007/s10905-010-9202-3

Tisserat, N., W. Cranshaw, D. Leatherman, C. Utley, and K. Alexander. 2009. Black walnut mortality in Colorado caused by the walnut twig beetle and thousand cankers disease. Plant Health Prog. (August 11, 2009). DOI 10.1094/PHP-2011-0630-01-BR

Tisserat, N., W. Cranshaw, M. Putnam, J. Pscheidt, C. A. Leslie, M. Murray, J. Hoffman, Y. Barkley, K. Alexander, and S. J. Seybold. 2011. Thousand cankers disease is widespread on black walnut, *Juglans nigra*, in the western

United States. Online Plant Health Prog. (June 30, 2011). DOI 10.1094/PHP-2011-0630-01-BR

(USDA) United States Department of Agriculture: Forest Service (FS) and Plant Protection and Quarantine (PPQ) 2016. Thousand cankers disease survey guidelines for 2016, April 2016, 11 pp. + Appendices. (https://www.aphis.usda.gov/plant_health/plant_pest_info/tcd/downloads/TCD_Survey_Guidelines.pdf)

Utley, C., T. Nguyen, T. Roubtsova, M. Coggeshall, T. C. Ford, L. J. Graulke, A. D. Graves, C. A. Leslie, J. McKenna, K. Woeste, et al. 2013. Susceptibility of walnut and hickory species to *Geosmithia morbida*. Plant Dis. 97: 601–607. (http://dx.doi. org/10.1094/PDIS-07-12-0636-RE)

Venette, R. C., R. D. Moon, and W. D. Hutchison. 2002. Strategies and statistics of sampling for rare individuals. Annu. Rev. Entomol. 47: 143–174.

Wiggins, G. J., J. F. Grant, P. L. Lambdin, P. Merten, K. A. Nix, D. Hadziabdic, and M. T. Windham. 2014. Discovery of walnut twig beetle, *Pityophthorus juglandis*, associated with forested black walnut, *Juglans nigra*, in the eastern U.S. Forests 5: 1185–1193. DOI 10.3390/f5061185

Williams, W. I., and I. C. Robertson. 2008. Using automated flight mills to manipulate fat reserves in Douglas-fir beetles (Coleoptera: Curculionidae). Environ. Entomol. 37: 850–856.

Zerillo, M. M., J. I. Caballero, K. Woeste, A. D. Graves, C. Hartel, J. W. Pscheidt, J. Tonos, K. Broders, W. Cranshaw, S. J. Seybold, and N. Tisserat. 2014. Population structure of *Geosmithia morbida*, the causal agent of thousand cankers disease of walnut trees in the United States. PloS one. 9: p.e112847.

Zhang, Y., L. Wang, K. Wu, K. A. Wyckhuys, and G. E. Heimpel. 2008. Flight performance of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) under different temperature and humidity regimens. Environ. Entomol. 37: 301–306.