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Source: Pan-Pacific Entomologist, 88(2):231-247. 2012.

Published By: Pacific Coast Entomological Society

DOI: <http://dx.doi.org/10.3956/2012-21.1>

URL: <http://www.bioone.org/doi/full/10.3956/2012-21.1>

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Diurnal flight response of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), to pheromone-baited traps in two northern California walnut habitats

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Abstract. The diurnal flight response of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), was assessed during two seasonal periods at two sites in northern California. Males and females flew primarily at dusk in response to aggregation pheromone-baited traps during late June/early July, and the percentage of beetles that flew between sunrise and late afternoon was positively correlated with the temperature at 6 AM of that day. Between late August and early November, the diurnal flight pattern was more varied and generally bimodal, though approx. 42 to 55% of the beetles still flew during the dusk period. During either period of the season, there was very little evidence that any flight occurred during the night. Between late August and late September, diurnal flight of both sexes during two-hour intervals was associated with temperature in a Gaussian manner with a threshold at 17–18 °C (62.6–64.4 °F) and peak activity at 23–24 °C (73.4–75.2 °F). The proportion of females in the catches of *P. juglandis* that responded to pheromone-baited traps ranged from 0.43 to 0.84 but did not vary throughout the day within a seasonal period.

Key Words. Aggregation pheromone, bark beetle, California, crepuscular flight activity, diurnal flight behavior, *Geosmithia morbida*, *Juglans hindsii*, northern California black walnut, pheromone-trapping, *Pityophthorus juglandis*, thousand cankers disease, walnut twig beetle.

INTRODUCTION

The walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae, *sensu* Wood 2007), is a native phloeophagous insect that has recently been associated with the newly described fungus, *Geosmithia morbida* (Kolařík et al. 2011). This insect-fungal complex, also known as thousand cankers disease (TCD), can be fatal to walnut trees and is responsible for the gradual deterioration of several species of black walnuts in the western United States over the past decade (Graves et al. 2009, Seybold et al. 2011, Tisserat et al. 2011). The beetle and pathogen have been introduced into Pennsylvania (2011), Tennessee (2010), and Virginia (2011) (Seybold et al. 2011). The pathogen infects walnut and butternut trees (both *Juglans* spp.) but not pecans and hickory (*Carya* spp.) (all Juglandaceae) (Utley et al. 2009).

There is no description of the flight behavior of the WTB or other aspects of its biology in the primary literature (Bright & Stark 1973; Bright 1981; Wood 1982; Wood & Bright 1992; Bright & Skidmore 1997, 2002). However, with the isolation of the male-produced aggregation pheromone of the WTB (Seybold et al. 2012a, b), we have a new tool to begin to understand various nuances of its flight behavior.

Temporal flight patterns of scolytids have been examined in short (i.e., diurnal) and long (i.e., seasonal) time scales (Daterman et al. 1965). On the diurnal scale, Daterman et al. (1965) examined the flight patterns of 10 species of bark and

ambrosia beetles in coniferous forests in western Oregon and found that half of the species flew during daylight hours, whereas the other half had crepuscular patterns of daily flight. One of the daytime fliers, *Trypodendron lineatum* (Oliv.), had a unimodal flight pattern in mid-May but a bimodal pattern in early June (Rudinsky & Daterman 1964). The diurnal flight patterns of other frequently trapped scolytids, *Gnathotrichus* spp. [likely *sulcatus* (LeConte) and *retusus* (LeConte)], were entirely crepuscular during these two months. The western pine beetle, *Dendroctonus brevicomis* LeConte, has been observed in flight only during daylight hours (Miller & Keen 1960). In northern California (from 23 to 29 June 1998), the daylight flight pattern of *D. brevicomis* in response to aggregation pheromone-baited traps was bimodal, with a small peak of flight occurring at 1200 h and a larger peak occurring at 1900 h (Fettig et al. 2004). A similar bimodal pattern during the day (with earlier morning flight) was described for several species of southern pine *Ips* and *Dendroctonus* responding to a naturally produced aggregation pheromone from beetle-infested cut logs (Vité et al. 1964). Some of the Ipini appear to have unimodal diurnal flight patterns [e.g., *I. typographus* L., Rudinsky et al. 1971; *I. duplicatus* (Sahlberg), Chen et al. 2009], whereas others have bimodal patterns [*Orthotomicus erosus* (Wollaston) and *Pityogenes calcaratus* Eichhoff, Mendel et al. 1991]. Other studies of diurnal behavior of bark and ambrosia beetles have relied more on observations of emergence than on flight responses to stimuli (Saunders & Knoke 1967, Novak 1976, Haack 1985).

In 2011, we studied the diurnal flight behavior of the WTB in response to its aggregation pheromone during two seasonal periods at two northern California locations. The objectives of the research were to determine the modality and time of maximum flight by WTB during two seasonal periods and whether there was a temperature threshold related to flight activity during the daylight period. We were also interested in determining whether or not the sex ratio of responding WTB changed in response to time and temperature. We reasoned that these characteristics of the behavior might be valuable information for an international monitoring survey for detecting WTB in new areas of North America (Seybold et al. 2012a).

METHODS AND MATERIALS

Study Sites. Diurnal flight studies of WTB were conducted from 23 June to 8 July 2011 in Solano Co., California and from 29 August to 7 November 2011 in Yolo Co., California. The Solano Co. site was in an orchard setting at the USDA National Clonal Germplasm Repository, *Juglans* Collection, located approx. 2.5 km SW Winters (UC-Davis/USDA ARS Wolfskill Experimental Orchards, 38°30'1.76" N, 121°58'39.83" W, approx. 41.5 m elev.). This germplasm collection contains 15 different species and hybrids of 24–29 yr-old *Juglans*; the primary species near the flight traps were *J. californica* S. Wats., *J. microcarpa* Berland, and *J. ailantifolia* Carr. The Yolo Co. site was in a native riparian stand of *J. hindsii* (Jeps.) Jeps. ex R.E. Sm., located along the north fork of Putah Creek in Davis (38°32'20.66" N, 121°44'21.42" W, approx. 16 m elev.).

Temperature Data at the Study Sites. For the Solano Co. site, hourly temperature data (23 June through 8 July 2011) were obtained from weather station #139, located approximately 100 m northwest of the trapping site, from the California Irrigation Management Information System, Department of Water Resources (CIMIS) website (<http://www.cimis.water.ca.gov/>). For the Yolo Co. site, hourly

temperature data (31 August through 7 November 2011) were obtained from the same web site linked to weather station #6 (38°32'8.78" N, 121°46'34.92" W, elevation 18.3 m), located approximately 2.9 km west of the trapping site. Additional data for the Yolo Co. site were obtained from the UC Davis Climate Station at the Campbell Tract (38°32'6.42" N, 121°46'24.17" W, elevation 18.3 m) from the UC Davis Climate Station website (http://atm.ucdavis.edu/weather/weather_station.php). Onsite temperature data for the Yolo Co. site were gathered bihourly from a thermometer (Fisher Scientific, Atlanta, Georgia) attached to a trap in the middle of the array, as well as electronically with a HOBO H8 Family Data Logger (Onset Computer Corp., Cape Cod, Massachusetts) located in the middle of the array and programmed to record data on 5 min intervals. Information from the HOBO data logger was downloaded by using Boxcar v3.7 software (Onset).

Flight Trapping. To assess the flight behavior of WTB, we used four-unit (Solano Co. site, $n = 8$ traps) and twelve-unit (Yolo Co. site, $n = 5$ traps) black plastic multiple funnel traps (Lindgren 1983) with a wet collecting cup (Contech Enterprises Inc., Delta, B.C., Canada). The cup was filled with propylene glycol-based recreational vehicle or marine antifreeze to a depth of 2.5 to 5 cm (Peak RV & Marine Antifreeze, UPC#7480400255, Old World Industries Inc., Northbrook, Illinois or Traveler RV & Multi-Purpose Antifreeze, UPC#4939402302, Tractor Supply Co., Brentwood, Tennessee). At both sites, traps were baited on the inside of the middle funnel with a proprietary formulation of the WTB aggregation pheromone (Seybold et al. 2012b). Traps at both locations were suspended from the top of a 3.05-m length of 1.27 cm inner diameter/1.91 cm outer diameter, thin-walled galvanized steel conduit pole [Wheatland Electrical Metallic Tubing (EMT), Issue No. TT-548 (or IT-548), UPC 8669202001 (Wheatland Tube Co., Sharon, Pennsylvania); or Allied EMT, Issue No. TT-540, UPC 9111102001 (Allied Tube and Conduit, Harvey, Illinois)]. When installed, the lid of the trap was about 20–22 cm from the top of the pole. The poles with attached traps were then slid over a 1.04 to 1.27 m rebar stake (1.27 cm diameter) that had been driven into the ground about 3–5 m from the base of *Juglans* trees.

Traps were emptied by filtering the contents in the antifreeze through conical paper/nylon paint strainers (Astro Pneumatic, Product No. AST-4583, Mt Airy, North Carolina, U.S.A., www.toolrage.com), which were then placed into Ziploc bags and processed in the laboratory. At the Solano Co. site, the eight traps were part of an experiment to test the efficacy of Rain·x glass treatment (Sophus Products, Houston, Texas) as a trap-surface lubricant (results to be reported elsewhere). These traps were emptied at approx. 0700 h and 1730 h (all times PDT) nearly each day between 23 June and 8 July 2011 (Table 1). At the Yolo Co. site, the five traps were placed specifically to assay for the diurnal flight behavior of WTB. They were emptied at two-hour intervals from 0600 h until 2000, 2100, or 2200 h each day between 31 August and 30 September 2011, and approx. 0800 and 1700 h each day between 13 October and 7 November 2011 (Table 1).

Sorting Trap Catches. WTB in the trap catches were separated from vegetative matter and other insects under a dissecting microscope [Zeiss Stemi 2000 stereomicroscope, 40× to 60× magnification (Fisher Scientific)], and the sexes were separated based on the pubescence on the female frons and minute spines/tubercles on the male elytral declivity (Bright 1981).

Data Handling and Statistical Analyses. Responses of males and females were recorded and analyzed separately, both as mean absolute trap catches per time

Table 1. Assay periods and diurnal intervals for assessing the flight response of *Pityophthorus juglandis* to pheromone-baited traps.

Assay Period	Dates (2011)	<i>n</i> ^a	Diurnal Time Points when Traps were Emptied
1-Solano Co.	23 June to 8 July	11	0700 and 1730 h
2-Yolo Co.	31 August to 30 September	17	0600, 0800, 1000, 1200, 1400, 1600, 1800, and 2000 h (sometimes at 2100 and 2200 h)
3-Yolo Co.	13 October to 7 November	9	0800 and 1700 h

^a Number of trapping intervals.

period as well as percentages of the trap catches during the daylight or dusk/overnight period. In general, the daylight period from one calendar day (i.e., 0600 to 1800 h, 0700 to 1730 h, or 0800 to 1700 h) was paired with the dusk and overnight period of that and the subsequent calendar day to form a 24 hr period for analysis.

Beetles caught in Solano Co. were compared between the daylight period (from 0700 to 1730 h) and the dusk/overnight period (from 1730 to 0700 h) by using generalized estimating equations (GEE) with dates as repeated measurements (PROC GENMOD in SAS) (SAS Institute Inc. 2010). The distribution of errors was modeled as Poisson and beetle catches were linked to their expected values with a log function. Separate analyses were conducted for males and females. Proportions of females caught during the day and night were normally distributed and analyzed with paired *t*-tests (PROC TTEST in SAS). To investigate the relationship between temperature (*T*; °C) and beetle catches, percentages of beetles caught during the daylight period were regressed against temperature at 0600 h, against the maximum daily temperature, and against temperature at 1800 h (PROC REG in SAS).

Beetles caught in Yolo Co. were compared between the daylight period (from 0600 to 1800 h, 31 August to 30 September, *n* = 22; from 0800 to 1700, 13 October to 7 November, *n* = 15) and the dusk/overnight period (from 1800 to 0600 h and from 1700 to 0800, respectively) (Table 1) with the same methods as described for the Solano Co. trap catches. Regression analyses between temperature at three points during the day and percentage of beetles caught during the daylight period were conducted as described above for the data from Solano Co., but separate analyses were carried out for the periods of 31 August to 30 September and 13 October to 7 November. Because we emptied the traps more frequently between 31 August and 30 September, we were also able to examine the data during this period over seven bi-hourly periods and one longer period during the scotophase (2000 to 0600 h). These data were analyzed by GEE to study the flight pattern during these shorter periods. If the overall null hypothesis that there were no significant differences in catches among treatments (i.e., the eight time periods) was rejected ($\alpha = 0.05$), then the least square means were separated by a Student *t*-test. For the bi-hourly data set, the distribution of errors was modeled as multinomial with beetle catches linked to their expected values with a cumulative logit function because the SAS program encountered an estimation problem when the distribution was modeled as Poisson. Proportions of females caught during the daylight and dusk/overnight periods were analyzed separately for the 31 August to 30 September and 13 October to 7 November periods [normally distributed data, paired *t*-tests (PROC TTEST in SAS)]. Only dates when females were caught both in the daylight and dusk/overnight

periods ($n = 17$ and 9 for 31 August to 30 September, and 13 October to 7 November, respectively) were included in the analyses. To examine the relationship between WTB trap catches and temperature between 31 August and 30 September, the total number of each sex of WTB caught during each interval of the day was regressed with mean temperature during that interval by using a Gaussian curve (i.e., $Y = a \cdot e^{-\frac{0.5(X - X_0)^2}{b}}$) in SigmaPlot Version 12.0 (Systat Software, Inc.). Mean temperature over each two-hr period was calculated based on measurements recorded every 5 min from the UC Davis Climate Station (Campbell Tract).

Voucher Specimens and Nomenclature. Voucher specimens of WTB adults from these studies were placed at the California Academy of Sciences, San Francisco, California. In this note, we have elected to use the original nomenclature for bark and ambrosia beetles (Coleoptera: Scolytidae) based on the argument presented in Wood (2007) and a more extensive treatment of the issue developed by D.E. Bright (personal communication), which is to be published in his third supplement to the world catalog of the Scolytidae and Platypodidae. In essence, morphological and fossil evidence of adult scolytids support 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.

RESULTS

Solano Co. Flight Study. A total of 4008 WTB were trapped during the 11 assay periods in the flight study between 23 June and 8 July 2011 at the Solano Co. site (Fig. 1A). The grand mean daily trap catches were 7.63 ± 1.32 males/trap/day and 37.92 ± 3.95 females/trap/day (8 traps per day and eleven 24 hr trapping periods, $n = 88$). The overall proportion of females among total beetles trapped was 0.83.

Significantly more beetles were caught during the dusk/overnight period in Solano Co., regardless of beetle sex (Male: $\chi_1^2 = 6.38$, $P < 0.05$; Female: $\chi_1^2 = 7.34$, $P < 0.01$) (Fig. 1B). When examined over each 24 hr period, the percentages of males and females that flew between 1730 h of one calendar day and 0700 h of the next calendar day were $89.1 \pm 6.0\%$ and $89.4 \pm 4.8\%$ (both $n = 11$), respectively (Fig. 1B). The proportion of female WTB trapped during daylight hours did not differ from the proportion trapped during dusk/overnight hours ($P > 0.05$) (Table 2). Temperature at 0600 h had a positive association with beetle catches during the daylight period (Table 3). The linear regression equations for both sexes had significant slopes. Neither the daily maximum temperature (both sexes) nor temperature at 1800 h (males) had a significant positive association with catch during the daylight period (Table 3). Quadratic regressions did not improve the fit of the data over linear regressions.

Yolo Co. Flight Study. A total of 1391 WTB were trapped in flight between 31 August and 7 November 2011 at the Yolo Co. site (Fig. 2). This included the 22 (31 August to 30 September) and 15 (13 October to 7 November) assay periods (1092 WTB), as well as the intervening time period when the traps were in place but emptied sporadically (299 WTB). The grand mean daily trap catches during the two assay periods were 1.83 ± 0.16 males/trap/day and 2.78 ± 0.24 females/trap/day (5 traps per day and thirty-seven 24 hr trapping periods, $n = 185$). The overall proportion of females among total beetles trapped was 0.59. During the combined late-summer and fall assay periods (31 August to 30 September, 13 October to 7

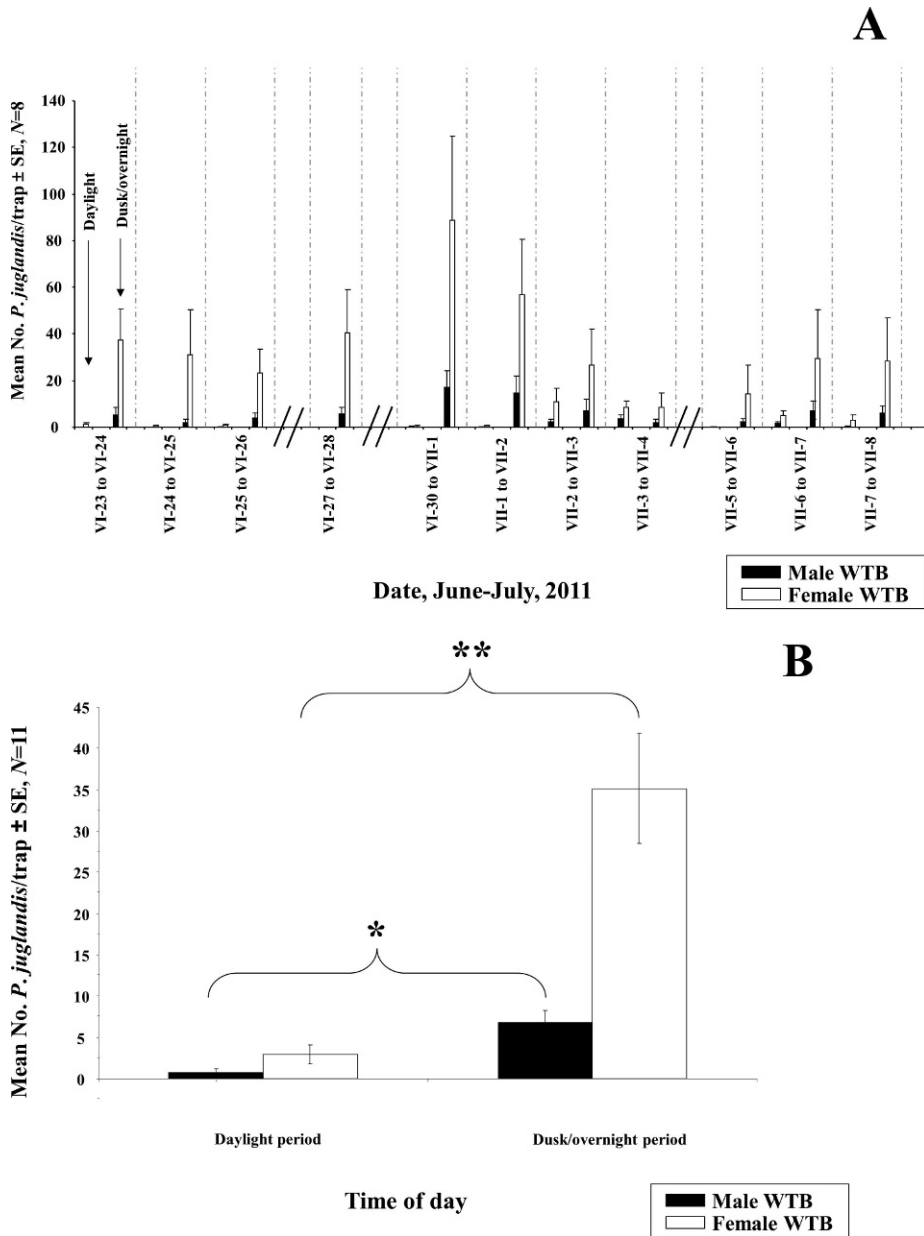


Figure 1. (A) Diurnal flight responses of *Pityophthorus juglandis* males (dark bars) and females (open bars) to eight, 4-unit Lindgren funnel traps baited with the *P. juglandis* aggregation pheromone, 23 June to 8 July 2011, Winters, Solano Co, California. Mean ($n = 8$) response of each sex during daylight and dusk/overnight periods over 11 days. Vertical dashed lines indicate 24 hr periods. Hash marks along the X-axis indicate breaks in continuous servicing of the traps. (B) Cumulative diurnal flight responses of *P. juglandis* males (dark bars) and females (open bars) ($n = 11$); * indicates a significant difference at $P < 0.05$; ** indicates a significant difference at $P < 0.01$, χ^2 test.

Table 2. Proportions of female *Pityophthorus juglandis* responding to pheromone-baited traps during different diurnal intervals.

Assay Period	Dates (2011)	<i>n</i> ^a	Proportion of Females		<i>t</i> -value	<i>P</i> -value
			Daylight	Dusk/Overnight		
1 ^b	23 June to 8 July	11	0.84 ± 0.04	0.84 ± 0.01	0.20	> 0.05
2 ^c	31 August to 30 September	17	0.61 ± 0.06	0.59 ± 0.05	0.24	> 0.05
3 ^d	13 October to 7 November	9	0.43 ± 0.12	0.52 ± 0.09	0.55	> 0.05

^a Number of trapping intervals.

^b Daylight (0700 to 1730 h); Dusk/overnight (1730 to 0700 h).

^c Daylight (0600 to 1800 h); Dusk/overnight (1800 to 0600 h).

^d Daylight (0800 to 1700 h); Dusk/overnight (1700 to 0800 h).

November), the number of male beetles ($1.09 \pm 0.14/\text{trap}/\text{day}$) caught during the daylight period did not differ from that of the dusk/overnight period ($0.76 \pm 0.11/\text{trap}/\text{day}$) ($\chi_1^2 = 1.39$, $P > 0.05$) (Fig. 2). The number of female beetles ($1.77 \pm 0.24/\text{trap}/\text{day}$) caught during daylight also did not differ from that of dusk/overnight ($1.14 \pm 0.14/\text{trap}/\text{day}$) ($\chi_1^2 = 2.50$, $P > 0.05$) (Fig. 2).

For 31 August to 30 September, when the data were examined twice daily over each 24 hr period, the majority of beetles flew between 1800 h of one calendar day and 0600 h of the next calendar day (Fig. 3A). The percentages of males and females that flew during this dusk/overnight period were $57.9 \pm 7.8\%$ and $53.7 \pm 8.1\%$ (both $n = 22$), respectively (Fig. 3B). These responses were not significantly different from those during the daylight period (χ^2 test, $P > 0.05$). Within the assay period, there appeared to be a marked shift in these percentages around 18–19 September after which both sexes flew in relatively greater numbers during the daylight period (Fig. 3A, black arrow below x-axis). The proportion (sex ratio) of female WTB trapped during daylight hours did not differ significantly from those trapped during dusk/overnight hours ($P > 0.05$) (Table 2). After examining all of the local and regional temperature data available to us for the Yolo Co. site, we found that the regional data from the UC Davis Climate Station at the Campbell Tract was nearly identical to the temperatures on site. Because this was a more complete data set, we opted to use it in our data analysis. Temperature at 0600 h had no association with WTB catch during the daylight period, but both daily maximum and temperature at 1800 h had a significant positive linear association with trap catch (Table 3).

For 13 October to 7 November, when the data were examined over each 24 hr period (Fig. 4A), the percentages of males and females that flew between 1700 h of one calendar day and 0800 h of the next calendar day were $40.6 \pm 10.7\%$ and $44.4 \pm 11.5\%$ (both $n = 15$), respectively (Fig. 4B). The trend of considerably higher relative flight during the daylight period continued as the fall proceeded. The proportion (sex ratio) of female WTB trapped during daylight hours did not differ significantly from those trapped during dusk/overnight hours ($P > 0.05$) (Table 2). Temperature at 0800 h (which was more relevant than 0600 h at this late seasonal period), the daily maximum temperature, and temperature at 1700 h were not associated linearly with trap catch during the daylight period (Table 3).

For the 31 August to 30 September assay period when the data were examined bi-hourly, beetle catches (591 WTB) differed significantly among the eight periods of

Table 3. Regression analyses of the daylight flight responses of *Pityophthorus juglandis* to pheromone-baited traps with three daily temperatures.

Assay Period	Time Point (temperature range, °C)	Sex	Regression Equation	P-value (slope)	Adj. r^2 -value
1-Solano Co.	0600 h (13.3–21.9)	M	$Y = -64.48 + 4.50X$	0.03	0.44
23 June to 8 July 2011	Daily maximum (28.9–36.8)	F	$Y = -51.27 + 3.69X$	0.02	0.47
		M	$Y = -108.70 + 3.63X$	0.16	0.21
	1800 h (27.3–34.9)	F	$Y = -90.82 + 3.07X$	0.12	0.24
		M	$Y = -116.06 + 4.08X$	0.06	0.34
2-Yolo Co.	0600 h (11.3–20.5)	F	$Y = -96.0 + 3.42X$	0.04	0.38
		M	$Y = -10.82 + 3.65X$	0.28	0.06
31 August to 30 September 2011	Daily maximum (26.8–38.5)	F	$Y = -28.41 + 5.16X$	0.13	0.11
		M	$Y = -159.74 + 5.88X$	< 0.01	0.32
	1800 h (20.8–33.3)	F	$Y = -214.56 + 7.60X$	< 0.01	0.50
		M	$Y = -135.59 + 6.12X$	< 0.01	0.37
3-Yolo Co.	0800 h (8.7–20.2)	F	$Y = -169.83 + 7.45X$	< 0.01	0.52
		M	$Y = 145.36 - 6.28X$	0.07	0.28
13 October to 7 November 2011	Daily maximum (22.4–36.8)	F	$Y = 145.60 - 6.60X$	0.07	0.26
		M	$Y = 80.70 - 1.03X$	0.81	0.01
	1700 h (19.8–28.5)	F	$Y = 112.99 - 2.40X$	0.59	0.03
		M	$Y = 46.86 + 0.26X$	0.95	0.0003
		F	$Y = 108.53 - 2.44X$	0.60	0.03

the day, irrespective of beetle sex (Male: $\chi_7^2 = 20.91$, $P < 0.01$; Female: $\chi_7^2 = 20.46$, $P < 0.01$) (Fig. 5A). Periods 0800–1000 and 1800–2000 h caught significantly more male and female WTB than the other periods (Fig. 5A). Periods 2000–0600, 0600–0800, and 1000–1200 h caught more beetles than periods 1200–1400, 1400–1600, and 1600–1800 h, when almost no beetles were caught (Fig. 5A). We looked particularly closely at the trap catches during the late twilight and scotophase (Figs. 5B, 5C, 5D, 5E). On nine occasions we emptied the traps at 2000 and 2100 h, and three of the 591 beetles (0.51%) were trapped during this interval (8, 13, and 19 September), whereas six of the 591 beetles (1.0%) were trapped between 2000 and 0600 h (15, 18, 19, and 30 September) of the next calendar day. We speculate that these beetles may also

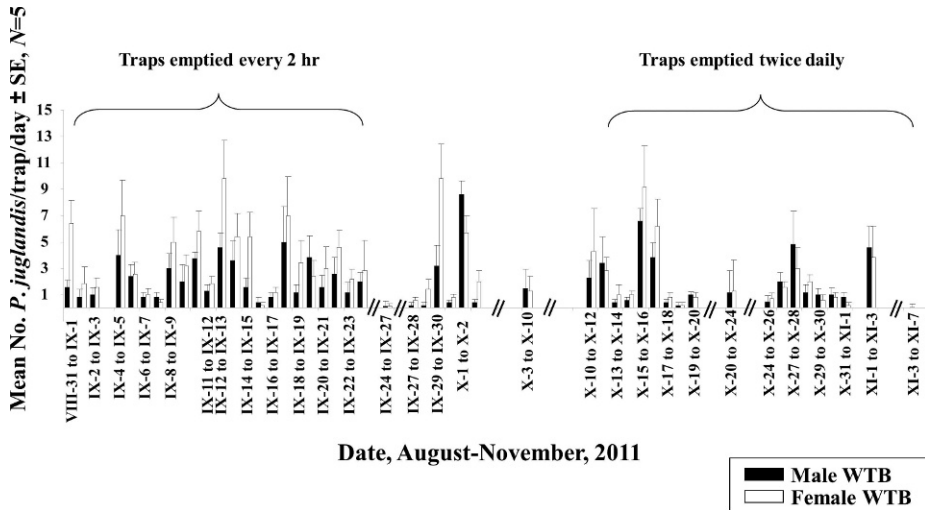


Figure 2. Daily flight responses of *Pityophthorus juglandis* males (dark bars) and females (open bars) to five, 12-unit Lindgren funnel traps baited with the *P. juglandis* aggregation pheromone, from the morning of 31 August to the morning of 7 November 2011, Davis, Yolo Co, California. Mean ($n = 5$) response of each sex during sixty-eight 24-hr periods. Brackets indicate the periods when traps were emptied every 2 hr throughout the day or emptied twice daily (at 0800 and 1700 h). For the intervening period (30 September to 12 October) traps were emptied intermittently because of relatively lower temperatures during that time. Data from the 2 hr-periods of trap capture were pooled for the purposes of this figure. Hash marks along the X-axis indicate breaks in continuous servicing of the traps.

have flown soon after 2000 h (we did not empty these traps again until 0600 h) or were on the traps at 2000 h but had not yet fallen into the cups when we emptied the traps. On several occasions we emptied or examined the traps at 2100 and 2200 h but never captured any WTB at 2200 h.

The relationship between WTB catches and temperature between 31 August and 30 September fit a Gaussian curve (male: $F_{2, 191} = 25.68$, $P < 0.001$; female: $F_{2, 191} = 27.24$, $P < 0.001$) (Figs. 6A, 6B). The parameters were $a = 4.30$, $b = 2.77$, $X_0 = 23.84$ for males and $a = 8.62$, $b = 2.55$, $X_0 = 23.3$ for females. The model suggests that beetles began to fly at 17–18 °C (62.6–64.4 °F) and the majority of beetles were trapped when the temperature was 23–24 °C (73.4–75.2 °F) (Figs. 6A, 6B).

Records. California: Solano Co., USDA National Clonal Germplasm Repository, *Juglans* Collection, approx. 2.5 km SW Winters, UC-Davis/USDA ARS Wolfskill Experimental Orchards, 38°30'1.76" N, 121°58'39.83" W, approx. 41.5 m elev., VII-1 to VII-2-2011, coll. S.J. Seybold, L.J. Nelson, ex: pheromone-baited funnel trap, diurnal flight study; Yolo Co., located along the north fork of Putah Creek in Davis, 38°32'20.66" N, 121°44'21.42" W, approx. 16 m elev., IX-3-2011, coll. S.J. Seybold, J.A. King, ex: pheromone-baited funnel trap #5, diurnal flight study, 1800 to 2000 h.

DISCUSSION

The rate of daily flight was much higher for both sexes of WTB during June/July at the Solano Co. site than during August/November at the Yolo Co. site. This could have been a true seasonal difference or could have been due to differences in population densities at the two sites. The Solano Co. orchard has a large number of

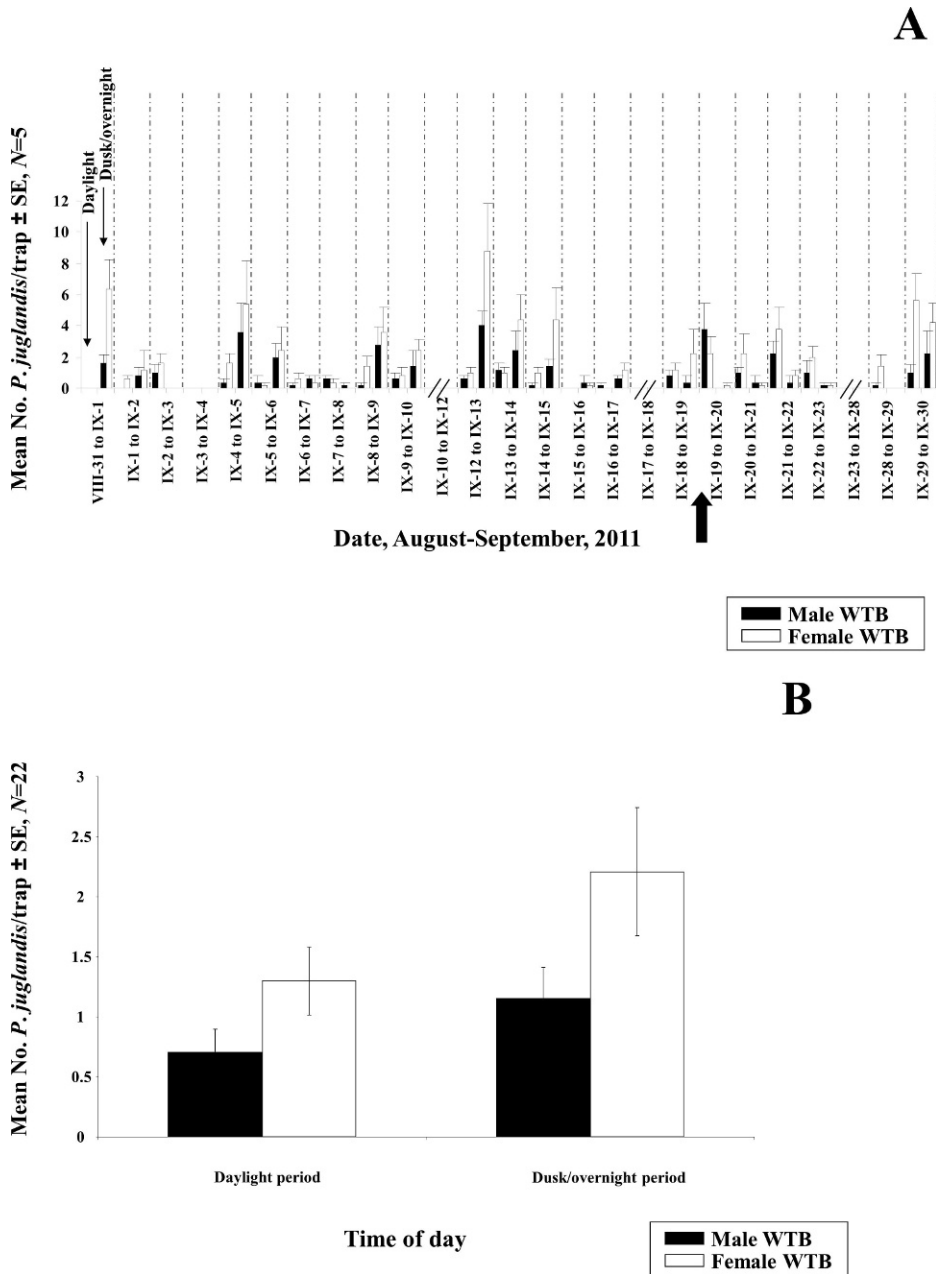


Figure 3. (A) Diurnal flight responses of *Pityophthorus juglandis* males (dark bars) and females (open bars) to five, 12-unit Lindgren funnel traps baited with the *P. juglandis* aggregation pheromone, 31 August to 30 September 2011, Davis, Yolo Co, California during daylight and dusk/overnight periods over 30 days (A). These data ($n = 5$) were pooled from trap catches from traps emptied every two hours during this time period (see Fig. 5 below). Vertical dashed lines indicate 24 hr periods. Hash marks along the X-axis indicate breaks in continuous servicing of the traps. Black arrow below x-axis indicates transition from dusk to daylight flight. (B) Cumulative diurnal flight responses of *P. juglandis* males (dark bars) and females (open bars) ($n = 22$). A chi-squared test showed no significant difference in the number of males or females that flew during the daylight vs. the dusk/overnight periods ($P > 0.05$).

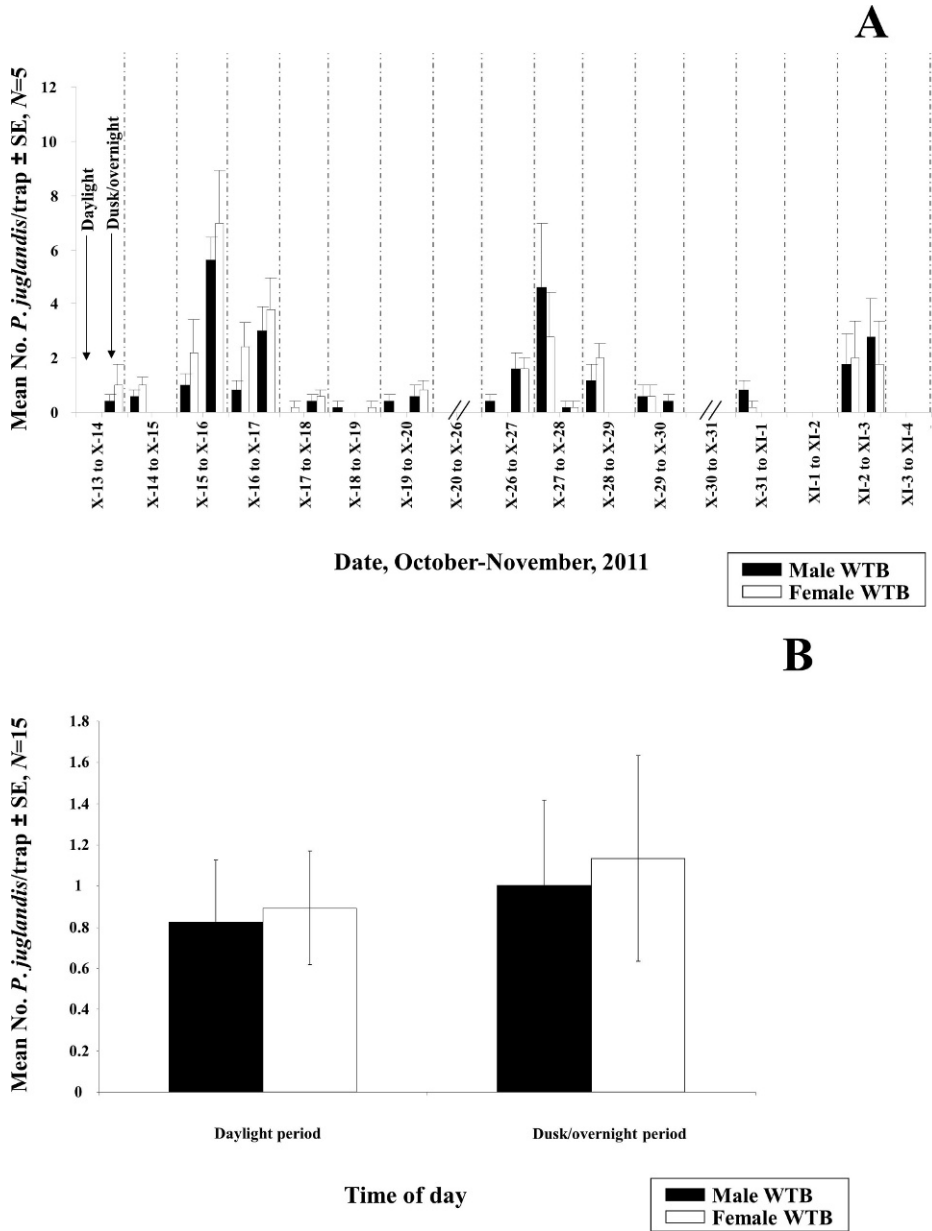


Figure 4. (A) Diurnal flight responses of *Pityophthorus juglandis* males (dark bars) and females (open bars) to 12-unit Lindgren funnel traps baited with the *P. juglandis* aggregation pheromone, 13 October to 3 November 2011, Davis, Yolo Co, California during daylight and dusk/overnight periods over 15 days ($n = 5$). Traps were emptied at approx. 0800 and 1700 h during this period. Vertical dashed lines indicate 24 hr periods. Hash marks along the X-axis indicate breaks in continuous servicing of the traps. (B) Cumulative diurnal flight responses of *P. juglandis* males (dark bars) and females (open bars) ($n = 15$). A chi-squared test showed no significant difference in the number of males or females that flew during the daylight vs. the dusk/overnight periods ($P > 0.05$).

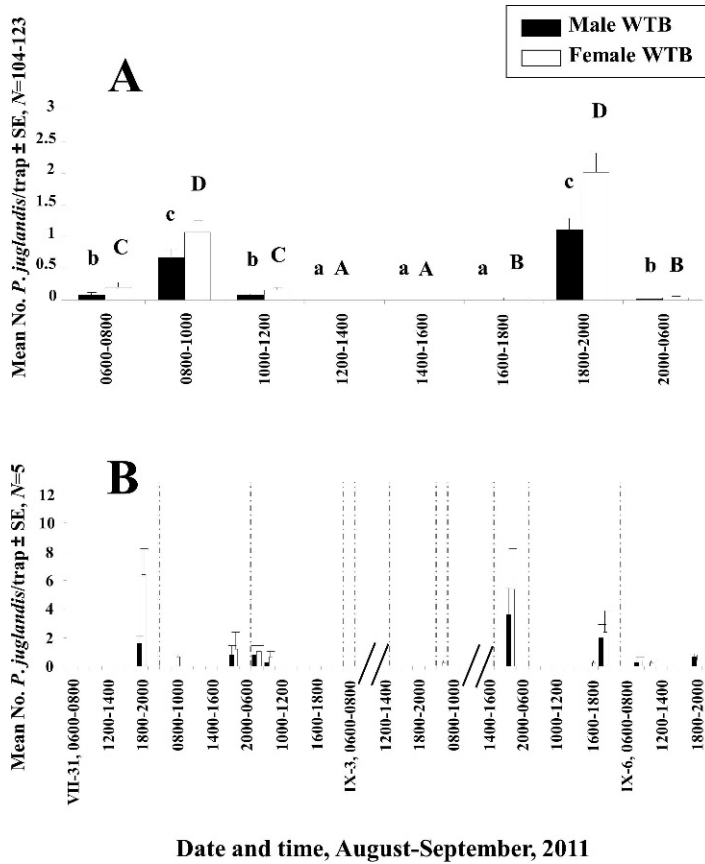


Figure 5. Diurnal flight responses of *Pityophthorus juglandis* males (dark bars) and females (open bars) during two-hr intervals to five, 12-unit Lindgren funnel traps baited with the *P. juglandis* aggregation pheromone, 31 August to 30 September 2011, Davis, Yolo Co., California. Mean responses of each sex during seven two-hr periods and one dusk/overnight period ($n = 119, 104, 110, 109, 114, 119, 127,$ and 123 for the eight periods beginning with 0600 to 0800 h), different lower (male) and upper (female) case letters represent significant differences among mean trap catches in intervals (A); mean responses of each sex between 31 August and 7 September (B); between 7 and 14 September (C); between 14 and 21 September (D); and between 21 and 30 September (E). Vertical dashed lines indicate 24 hr periods. Hash marks along the X-axis indicate breaks in continuous servicing of the traps.

dead and dying *J. californica* that likely provide good breeding habitat for WTB, whereas the Yolo Co. site contains only a few, large *J. hindsii*, some of which have dying branches.

We assessed the trap catches just after daybreak and then again just before dusk in Solano Co. (23 June–8 July, 0700 h and 1730 h) and in Yolo Co. (31 August–30 September, 0600 h and 1800 h; 13 October–7 November, 0800 and 1700 h). When traps were emptied during these periods, the majority of WTB in Solano Co. (approx. 90%) flew during the dusk/overnight period (trap catches emptied just after daybreak) (Fig. 1B). This pattern shifted somewhat when the early morning temperatures were elevated [Fig. 1A, e.g., at 0600 h on 2 July = 17.5 °C (63.5° F), at 0600 h on 3 July = 18.4 °C (65.1° F)]. At the Yolo Co. site, we emptied the traps

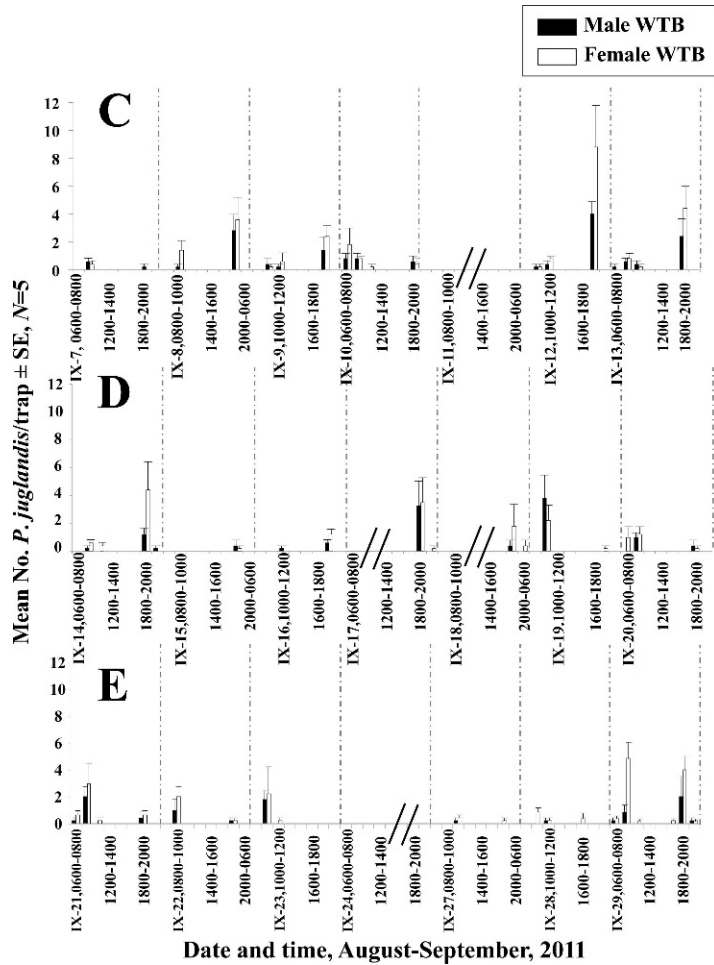


Figure 5. Continued.

every 2 hr beginning at 0600 h and continued until darkness (2000 or 2100 h) (31 August–30 September) and then pooled these catches numerically to construct daylight (0600 to 1800 h) and dusk/overnight (1800 to 0600 h) periods. In August/September, the majority of the flight (approx. 55%) was again during the dusk/overnight period (Fig. 3B). Later in the fall (13 October–7 November), the flight during the dusk/overnight period was less pronounced (approx. 42%) (Fig. 4B). When we examined the 2 hr trap catches from August/September, the largest catches were between 1800 and 2000 h. It was rare that WTB were in the traps when they were emptied at 2100 h or at 0600 h during the following day. This underscores that the flight of WTB during the summer and early fall was primarily crepuscular but likely never nocturnal. As the fall season proceeds, it appears that WTB flies at earlier times during the day (Figs. 3B, 4B, 5B, 5C, 5D).

Several workers have used pheromone-baited traps to assess the diurnal flight responses of bark beetles (Mendel et al. 1991, Fettig et al. 2004, Chen et al. 2009). These studies have tended to be somewhat abbreviated, likely because of the labor involved in servicing the traps at frequent intervals. Mendel et al. (1991) found that

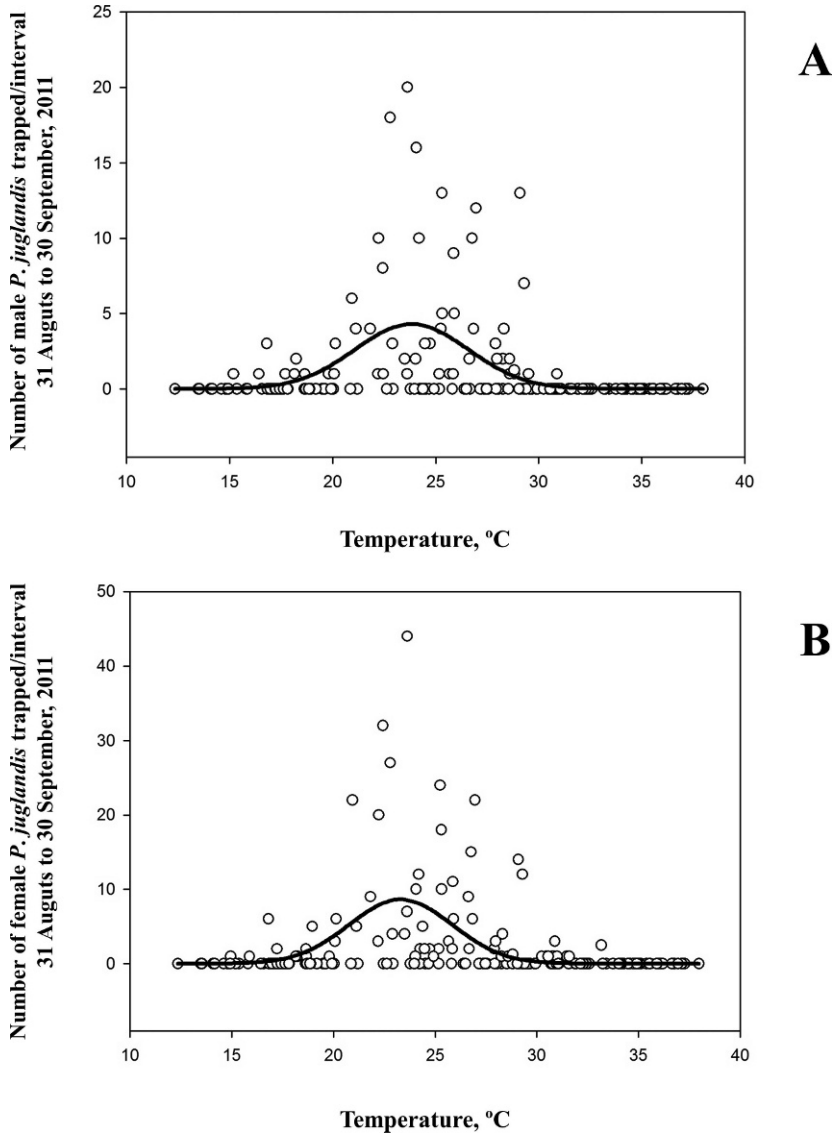


Figure 6. Gaussian curve ($Y = a \cdot e^{-\frac{(X - X_0)^2}{2b}}$) fit of WTB catches during each of eight trapping intervals against mean temperature (T; °C) of that interval for male (A; $F_{2, 191} = 25.68$, $P < 0.001$) and female (B; $F_{2, 191} = 27.24$, $P < 0.001$) by using SigmaPlot Version 12.0 (Systat Software, Inc.). Catches represent the sum of five trap catches. The parameters a , b , and X_0 were 4.30, 2.77, and 23.84 for males, and 8.62, 2.55, and 23.30 for females. WTB were caught each day during two-hour intervals from 0600 until 2000 h, and then between 2000 and 0600 h of the following day (final trapping interval) by using 12-unit Lindgren funnel traps baited with the aggregation pheromone between 31 August and 30 September 2011, Davis, Yolo Co., California.

the modalities of the diurnal flights of *O. erosus* and *P. calcaratus* varied with season. In winter, the flights were unimodal during daylight hours; in spring, flights were bimodal (late morning, early evening); and in summer, flights were bimodal (early morning, after dusk). There appeared to be some evidence of flight during the

scotophase with this species during the summer period. Whether the trends reported by Mendel et al. (1991) pertain completely throughout these seasonal periods is uncertain because the work was only conducted over 3-day intervals in each of the seasonal periods. In assaying the diurnal response of *D. brevicomis* to pheromone-baited traps during a one-wk period in June, Fettig et al. (2004) found that no *D. brevicomis* were trapped after 2100 and before 0800 h on the subsequent day. Miller & Keen (1960) also did not report any nocturnal flight of this species. Fettig et al. (2004) demonstrated that most of the *D. brevicomis* were trapped between 1700 and 2000 h (i.e., dusk). Chen et al. (2009) reported the unimodal diurnal flight response of *I. duplicatus* to its pheromone based on assays from two 3-day periods (early June and late July). They characterized a broad peak of flight during mid- to late-afternoon but did not find any evidence for flight during the scotophase.

In summer, it appears that WTB has a primarily unimodal (dusk) flight pattern, though bimodality (early morning and dusk) can be triggered on days with extremely high temperatures. This mirrors the summer flight patterns reported for *O. erosus* and *P. calcaratus* (Mendel et al. 1991). In fall, the flight pattern of WTB appears to be primarily bimodal (morning, dusk) (Figs. 3A, 4A, 5A) with increasing numbers of beetles flying earlier in the day as the season progresses. This trend also seems to match what Mendel et al. (1991) reported, whereby the winter season the flights of *O. erosus* and *P. calcaratus* were entirely unimodal. Additional research should be conducted to provide a full season perspective to the study of the diurnal flight behavior of WTB.

Various authors have inferred diurnal flight activity for bark or ambrosia beetles from emergence patterns from cut infested host material. A limitation of this approach is that emergence does not necessarily reflect immediate flight. This work was likely pursued because of the unavailability of an aggregation pheromone to assess flight directly. With *Ips calligraphus* (Germar), Haack (1985) reported that females emerged earlier than males and that the emergence activity of both sexes varied with the season. Specifically, both sexes had a unimodal emergence pattern during the late afternoon (1500 to 1700 h EST, spring; 1400 to 1700 h, fall); during mid-day (1200 to 1400 h, winter), but they had a bimodal emergence pattern (0700 to 0900 h and 1700 to 1900 h) in summer. Saunders & Knoke (1967) reported a primarily crepuscular emergence pattern for the ambrosia beetle, *Xyleborus ferrugineus* (F.), from caged trunk sections of cacao, *Theobroma cacao* L., whereas Novak (1976) reported that 80% of the emerging population of the ash bark beetle, *Hylesinus fraxini* (Panz.), emerged between 0930 and 1530 h over a 12-day period. Only five individuals (5 of 908) emerged during the night.

Future work on the diurnal flight responses of WTB should consider the interaction of light intensity and temperature in triggering flight. This has been suggested and investigated by others in relation to other scolytids (Daterman et al. 1965, Rudinsky et al. 1971). We noticed during data collection on particularly hot days, that the morning flight would typically occur between 0600 and 1000 before the onset of high daily light intensity. Thus, we suspect that there may be an interaction between temperatures ranging from 16 to 19 °C (60.8 to 66.2 °F) and low light intensity that may trigger flight of this beetle. Other physical factors such as wind speed (Mendel et al. 1991) or barometric pressure (Lanier & Burns 1978) could also be investigated as interacting influences with temperature and light on the diurnal flight habits of WTB.

ACKNOWLEDGMENTS

We dedicate this manuscript to the late Richard L. Penrose. “Dick” was a good friend and colleague, whose enthusiasm for the detection of invasive bark beetles and woodborers was contagious and stimulating to those around him. From 2004 through 2008, Dick had a big impact on the direction of our research by helping us re-focus from the chemical ecology of native forest insects to using the tools of chemical ecology to detect invading species in urban and peri-urban forest environments. He had a strong, positive influence on the research activities of postdoctoral associates, Jana C. Lee and Deguang Liu, who worked with us on invading elm and pine bark beetles. Dick also assisted with countless identifications of new bark beetles and longhorned beetles. Through his ever perceptive “nose” for new problems, from 2003–2007 Dick did some focused early modern collecting of walnut twig beetles in California. Both from funnel trap catches in his annual CAPS surveys and through rearing of infested walnut, Dick’s efforts presaged the current bloom in research on walnut twig beetle, which includes the discoveries reported in this paper. The authors would also like to thank Stacy Hishinuma for assistance with field work and Andrew Graves for assistance with literature retrieval and a helpful review of the manuscript. Funding for this work was provided by the USDA Forest Service, Pacific Southwest Research Station, and by grants from the USDA Forest Service, Washington Office Forest Health Protection and USDA APHIS CPHST administered through research agreements #10-CA-11272172-055 and 10-JV-11272172-092 between the USDA FS PSW Station and the UC-Davis Department of Entomology. We thank John Preece and Howard Garrison (both USDA ARS) for facilitating access to the Wolfskill orchard site.

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Received 22 Mar 2012; Accepted 6 June 2012 by F. W. Shockley; Publication date 14 Sep 2012.