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ABSTRACT Traps baited with pheromones are used to monitor the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), populations in flour mills to aid in making pest management decisions, but the factors that influence *T. castaneum* movement are not fully understood. We investigated the impact of photoperiod, light intensity, temperature, and relative humidity on flight initiation. The percentage of adults initiating flight reached a maximum at 30–35°C, and then fell to zero at 22.5 and 45°C. Only 2% of beetles flew in complete darkness, and the number of beetles initiating flight increased to 41% under 18 h of light and then decreased slightly to 37% under 24 h of light. Rates of flight initiation did not vary with light intensities from 1,784 to 4,356 lux or relative humidities from 25 to 85%. Thus, temperature and photoperiod are the main abiotic factors tested that impact flight initiation in *T. castaneum*, which have broad ranges of temperatures and photoperiods over which they can fly. The current results should be useful in helping to interpret trap catches based on abiotic conditions during the trapping period, and the results should be useful in helping to understand *T. castaneum* movement outside grain storages and processing facilities and their potential to infest structures.

KEY WORDS light intensity, photoperiod, relative humidity, temperature, *Tribolium castaneum*

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is the main pest of flour mills in much of the world. Traps baited with pheromones are used to monitor *T. castaneum* populations in flour mills to aid in making pest management decisions (Campbell et al. 2010), but the factors that influence *T. castaneum* capture are not fully understood. Both flying and walking *T. castaneum* may be attracted to traps. The relative contribution of flight versus walking dispersal can have important implications on movement within mills (Semeao et al. 2012, 2013) and the outside environment (Ridley et al. 2011), capture in pheromone-baited traps, and the potential for reinfestation after treatments (Boon and Ho 1988, Campbell et al. 2010).

Perez-Mendoza et al. (2011a,b) reported on biotic factors that affect flight initiation in *T. castaneum.* Slightly more adults flew when reared at high densities than when reared at low densities. Rates of flight initiation did not differ with sex or age of adults when beetles were up to 20 d old. But, when older beetles were tested (up to 165 d old), flight initiation decreased as age of the beetles increased. Rates of flight initiation also did not differ with duration of starvation for up to 5 d, but rate of flight initiation decreased as duration of starvation increased for longer than 5 d and no beetles starved for >18 d flew. The rate of flight initiation decreased as the amount of food in flight chambers increased. When no food was present in the flight chambers, more mated beetles flew than virgin beetles, but there was no difference in flight initiation between virgin and mated beetles when food was present. When multiple *T. castaneum* were placed in the same flight chamber, they were less likely to fly when placed with members of the opposite sex than when placed with members of the same sex.

There are few studies that describe the effects of abiotic factors on flight initiation in *T. castaneum.* Throne and Cline (1994) caught *T. castaneum* on sticky traps placed around grain storage areas during periods when maximum temperatures at nearby weather stations did not exceed 17°C, indicating that the minimum temperature for flight might be as low as 17°C. Cox et al. (2007) reported that the minimum temperature for flight in laboratory studies was 25°C. We report here on the effects of temperature, relative humidity (RH), photoperiod, and light intensity on flight initiation of *T. castaneum.*

Materials and Methods

Insects. *T. castaneum* used in the study were collected from a midwestern flour mill in November 2001. They were reared on a diet of 95% by weight wheat

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flour and 5% brewer's yeast at 30°C, 75% RH, and a photoperiod of 12:12 (L:D) h.

Flight Chambers. Flight initiation was assessed in flight chambers as previously described by Perez-Mendoza et al. (2011a). The chamber consisted of the bottom half of a petri dish (60 mm diameter by 20 mm high) with the inner wall coated with sticky material (Sticky Stuff, Olson Products, Medina, OH) inverted over another petri dish bottom (base dish) of the same dimensions with the walls coated with Teflon PTFE 30 (DuPont, Wilmington, DE) to prevent insects from walking up the sides of the base dish. A small paper platform with an upwardly projecting triangle was placed on the floor of the base dish so that a beetle could climb up the platform and fly off. Flying beetles could be caught on the sticky material on the upper dish and be easily counted without opening the flight chamber.

Individual beetles, which had not been starved before tests, were placed in the base dishes without food, and then the base dish was covered with the coated petri dish. The flight chambers were placed on a tray and held in the laboratory in the dark for 60 min to let the insects adjust to the new environment before being placed at test conditions. No insects were found adhered to the sticky material during this 60-min holding period. The number of adults that adhered to the sticky bottom of the dish was counted after 72 h and was considered to represent the number of individuals initiating flight.

Photoperiod Bioassays. The effects of photoperiod on flight initiation were tested in individual environmental chambers (model I-36VL, Percival Scientific Inc., Perry, IA) maintained at 28°C, 65% RH, and different photoperiods. Photoperiods tested were 24, 18, 12, 6, and 0 h light during a 24-h period. Fifty unsexed 30–33-d-old adults were tested in four replications of each treatment for a total of 200 insects for each photoperiod. No food was provided to the adults during the flight tests.

Light Intensity Bioassays. The effects of three light intensities (4,356 ± 390, 3,092 ± 193, and 1,784 ± 135 lux) on flight initiation were tested. These flight intensities were chosen because they were similar to those used in other studies on stored-product insects (e.g., Dowdy 1994) and because few insects initiated flight in the dark in the photoperiod study described in Photoperiod Bioassays section. For reference, lux levels at noon on a sunny day can be 110,000 in direct sunlight, 20,000 in the shade, and 10,000 on an overcast day (http://en.wikipedia.org/wiki/Daylight accessed on 6 November 2013). The experiment was conducted in a wood arena (120 cm in length by 120 cm in width by 23.5 cm in height) painted white (Zinsser Bulls Eye 1-2-3 primer, Rustoleum, Vernon Hills, IL) and placed in a walk-in environmental chamber (model CTH-1620, Percival Scientific Inc., Perry, IA) maintained at 28°C, 65% RH, and a photoperiod of 24:0 (L:D) h. Twenty flight chambers were placed inside each of nine polystyrene storage boxes (13.2 cm in height by 40 cm in length by 22 cm in width; McMaster-CARR, Santa Fe Springs, CA). The lower light intensities were obtained by placing six 120-cm-length fluorescent light bulbs 80 cm above the arena and covering the polystyrene storage boxes containing the flight chambers with sheets of paper. Three of the polystyrene boxes were not covered with paper to obtain the 4,356 lux light intensity treatment, while three of the polystyrene boxes were covered with one sheet of white paper (Art Kraft Duo-Finish paper; 45 cm in length by 25 cm in width) to obtain the 3,092 lux light intensity treatment and three of the polystyrene boxes were covered with three sheets of white paper to achieve the 1,784 lux light intensity treatment. Light intensity was measured at the floor of each polystyrene box (EasyView Light Meter model EA33, EXTECH Instruments, Nashua, NH). Sixty unsexed adults (22–25 d old in Replication 1 and 25–28 d old in Replication 2) were tested in each of the two replications of each treatment for a total of 120 insects for each light intensity treatment. No food was provided to the adults during the flight tests.

RH Bioassays. The effects of RHs from 25 to 85% (every 10%) on flight initiation were tested in seven individual environmental chambers (Percival I-36VL) maintained at 32.5°C and a photoperiod of 12:12 (L:D) h. Flight chambers for this study were slightly modified by adding several drops of silicon on the upper edge of the base petri dish to create a small gap between the base and top petri dishes to allow for air exchange and moisture equilibration. Fifty unsexed 30–33-d-old adults were tested in each of four replications per treatment for a total of 200 insects for each RH. No food was provided to the adults during the flight tests.

Temperature Bioassays. The effects of temperatures from 20 to 45°C (every 2.5°C) on flight initiation were tested in environmental chambers (Percival I-36VL) maintained at 65% RH and a photoperiod of 14:10 (L:D) h. Fifty unsexed 31–34-d-old adults were tested in each of four replications per temperature for a total of 200 insects for each temperature. No food was provided to the adults during the flight tests.

Data Analysis. The individuals within each replicate were grouped together to calculate a percentage of individuals that initiated flight, and then we analyzed those percentages using the analysis of variance (ANOVA) procedure in SigmaPlot (SYSTAT Software Inc. 2011). When ANOVA showed statistically significant differences among treatments, we fit equations to the data using TableCurve 2D (SYSTAT Software Inc. 2002).

Results

Photoperiod. Only 2% of beetles flew in complete darkness, and the number of beetles initiating flight increased to 41% under 18 h of light and then decreased slightly to 37% under 24 h of light ($F = 31.6$; df = 4, 15; $P \leq 0.001$; Fig. 1; Table 1).

Light Intensity. Percentage adults initiating flight did not differ with light intensity ($F = 0.2$; df = 2, 3; $P = 0.81$; mean ± SE = 52.5 ± 5.8 under 1,784 lux,

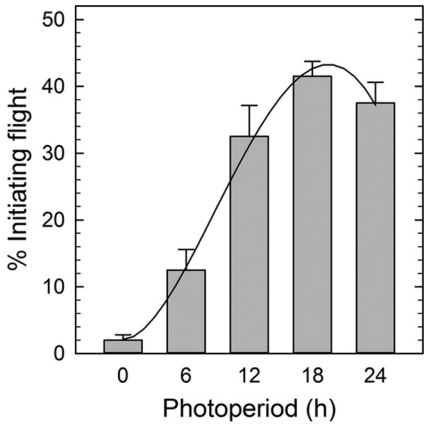

Fig. 1. Observed percentage *T. castaneum* adults initiating flight at photoperiods ranging from 0 to 24 h (bars), and percentage initiating flight predicted using equation in Table 1 (line).

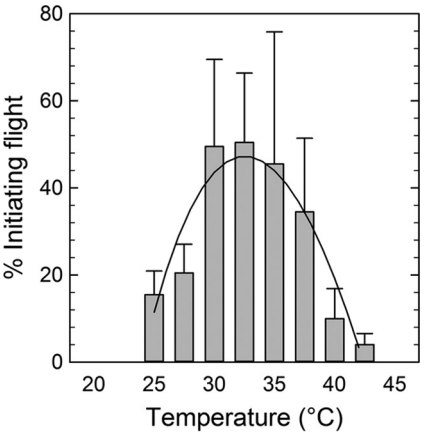

Fig. 2. Observed percentage *T. castaneum* adults initiating flight at temperatures ranging from 20 to 45°C (bars), and percentage initiating flight predicted using equation in Table 1 (line).

52.5 ± 7.5 under 3,092 lux, and 47.5 ± 4.2 under 4,356 lux).

Relative Humidity. Percentage adults initiating flight did not differ with RH ($F = 1.1$; df = 6, 21; $P = 0.42$; mean ± SE = 0.52 ± 0.05, 0.47 ± 0.03, 0.51 ± 0.05, 0.48 ± 0.03, 0.47 ± 0.04, 0.44 ± 0.05, and 0.40 ± 0.01 at 25, 35, 45, 55, 65, 75, and 85% RH, respectively).

Temperature. Percentage adults initiating flight reached a maximum of ≈50% at 30–35°C, and then fell to 0 at 22.5 and 45°C ($F = 23.7$; df = 10, 33, $P \leq 0.001$; Fig. 2; Table 1).

Discussion

Maximum percentage flight initiation was ≈50%, which is typical for this species (Perez-Mendoza et al. 2011a,b). Temperature is generally considered one of the main abiotic factors governing flight initiation in insects, and that is also the case for *T. castaneum.* Cox et al. (2007) previously reported that 25°C was the minimum temperature for flight of *T. castaneum* in laboratory studies, but Throne and Cline (1994) had captured *T. castaneum* on flight traps during periods when the maximum temperature recorded at nearby weather stations was 17°C. Our results were the same as Cox et al. (2007), with no *T. castaneum* initiating flight at temperatures <25°C. In the field study by Throne and Cline (1994), weather data were obtained from nearby weather stations, so temperatures at the trap site may have differed. Also, insects often sun themselves in cold weather, allowing them to warm up enough to fly even on cold days. Cox et al. (2007) reported that the greatest number of *T. castaneum* flew at 30°C, whereas we found that the percentage *T. castaneum* initiating flight was similar from 30 to 35°C. In general, stored-product beetle pests, such as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and the larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae), can initiate flight at temperatures between 17.5 and 25°C (Dowdy 1994, Cox and Dolder 1995, Fadamiro and Wyatt 1995, Cox et al. 2007, Fardisi and Mason 2013), maximum rates of flight initiation occur from 25 to 35°C (Dowdy 1994, Cox and Dolder 1995, Fadamiro and Wyatt 1995, Cox et al. 2007, Fardisi and Mason 2013), and insects are unable to fly at temperatures >32.5–40°C (Fadamiro and Wyatt 1995, Cox et al. 2007). However, we found that rates of flight initiation for *T. castaneum* were high at 37.5°C, and some beetles flew at temperatures as high as 42.5°C.

Photoperiod also had a great effect on flight initiation in *T. castaneum*, with high rates of flight initiation occurring when there were 12 h of light or more during the day, so the differences in flight initiation with day length are probably due to the reduced time available for flight. Few *T. castaneum* flew in the dark. These results are similar to those of Aslam et al. (1994) for *R. dominica*, although the reduction in flight as hours of light per day decrease is much more pronounced for *T. castaneum.* Boon and Ho (1988) reported *T. castaneum* at a rice warehouse in Singapore had peak flight activity between 1700 and 2100 hours (at 31°C, 84.5% RH, and 1,076 lux).

We found that rate of flight initiation did not vary with light intensity or RH. Perttunen and Tuula (1970) reported that the scolytid beetle, *Blastophagus piniperda* L., initiated flight at much higher rates at high light intensity when tested for a short period of time (15 min), but that flight initiation increased consid-

Table 1. Parameters (±SE) for equations describing effects of abiotic factors (X) on percentage flight initiation (Y) of *T. castaneum*

Abiotic factor	*a*	*b*	*c*	r^2
Photoperiod[a]	2.11 ± 2.38	0.541 ± 0.0684	−0.0980 ± 0.0138	0.89
Temperature[b]	−2,311 ± 300.9	−72.52 ± 9.100	827.1 ± 105.0	0.70

[a] Equation is of the form $Y = a + bX^2 + cX^{2.5}$. Lack-of-fit: $F = 0.2$; df = 2, 15; $P = 0.826$.

[b] Equation is of the form $Y = a + bX + cX^{0.5}$. Lack-of-fit: $F = 2.5$; df = 5, 24; $P = 0.056$.

erably at low light intensities over time. Flight initiation in *R. dominica* also has been reported to not be affected by RHs from 25 to 75% (Dowdy 1994, Fadamiro and Wyatt 1995).

Thus, temperature and photoperiod are the main abiotic factors tested that impact flight initiation in *T. castaneum*, which have broad ranges of temperatures and photoperiods over which they can fly. However, 25°C does appear to be the lower limit for flight initiation; therefore, captures of flying *T. castaneum* at lower temperatures probably result from flight initiation from warmer microhabitats or from individuals sunning themselves. On the surface of grain in a bin, *T. castaneum* would be expected to be exposed to normal light cycles, although the light would be of low intensity. So, based on our results, they should be able to fly readily. Light availability in a flour mill would vary greatly depending on location, but conditions for flight are readily available in a flour mill. A better understanding of the role of flight in dispersal within mills will be useful in understanding spatial patterns of distribution and identification of sources of infestation (Semeao et al. 2012, 2013). The current results should be useful in helping to interpret trap catches based on abiotic conditions during the trapping period, and the results should be useful in helping to understand *T. castaneum* movement outside grain storages and processing facilities and their potential to infest structures (Boon and Ho 1988).

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