

Tethered and Untethered Flight by *Lygus hesperus* and *Lygus lineolaris* (Heteroptera: Miridae)

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ABSTRACT We compared the flight behavior of *Lygus hesperus* Knight and *Lygus lineolaris* (Palisot de Beauvois) relative to age, sex, and time of day by using tethered (flight mills) and untethered flight (vertical flight chamber) assays. Both species and sexes initiated flights throughout the day, and flight mill assays recorded flights throughout the night. For both species and flight systems, most flights were <5 min in duration, with longest flights occurring from 1 to 3 wk of age. Number of flights and flight duration were influenced by age and sex for tethered individuals and by sex for untethered individuals. Species differences were less apparent, but tethered *L. lineolaris* had more sustained flights (individual flights >5 min) that were of longer cumulative duration compared with *L. hesperus*. The longest flights were obtained with flight mills and were 17-18 times longer than the longest flight (22 min) in the flight chamber. Determination of flight periodicity, throughout the day and night, was only possible for tethered insects, and females exhibited more distinct periodicities for sustained flights than males. For *L. hesperus* females, sustained flights followed a diurnal to crepuscular periodicity, whereas sustained flights by *L. lineolaris* females were nocturnal. No significant correlations were found between egg load and any of the flight parameters when grouped by species, but there was a positive correlation between the number of spermatophores and several of the flight parameters for female *L. hesperus*. In the vertical flight chamber, takeoffs began at low light levels and were always higher for *L. lineolaris* than *L. hesperus*. Rates of climb toward the skylight cue were ≈ 50 cm/s, indicating a capacity for strong, self-directed flight by both species.

KEY WORDS western tarnished plant bug, tarnished plant bug, flight mill, vertical flight chamber, flight behavior, egg load

IN NORTH AMERICA, *Lygus hesperus* Knight and *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae) are among the most important plant bug pests of agricultural and horticultural crops. *L. hesperus* is a perennial pest of cotton in the western United States (Ellsworth and Jones 2001), and *L. lineolaris* is becoming a primary pest of cotton in the southeastern United States, where the use of Bt cotton for lepidopteran pests and the success of boll weevil eradication programs have lessened the importance of these insects (Layton 2000). Both *Lygus* species are polyphagous, with 142 host plants listed for *L. hesperus* and 385 listed for *L. lineolaris* (Snodgrass et al. 1984, Young 1986, Schwartz and Foottit 1998). Their feeding activity causes abscission of reproductive structures, destroys plant terminals, and reduces plant quality and yield (Strong 1970, Leigh 1976, Mauney and Hen-

neberry 1984, Leigh et al. 1988, Williams and Tugwell 2000). *L. hesperus* is confined to the western United States and southwestern Canada, whereas *L. lineolaris* is found throughout North America. These differences in geographic distributions could be related to inherent variations in these two species flight propensities, which could have important implications in developing management strategies.

Stewart and Gaylor (1994) examined the tethered flight behavior of *L. lineolaris* and showed that age, reproductive status, and sex played an important role in the timing and duration of flights by this species. Little else is known about the flight capacity of either species or of the factors that influence these insect's tendencies to disperse. In this study, our primary objectives were to compare the flight capacity of *L. hesperus* and *L. lineolaris*, as well as two systems that have been used to study tethered and untethered flight in insects. We examined the effect of insect age, sex, reproductive and mating status, and time of day on flight propensity and duration for each species. We also used a vertical flight chamber to study responses to varying light intensities and to compare rates of climb between the species.

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Materials and Methods

Insect Rearing and Maintenance. *L. hesperus* colonies were started from collections made in alfalfa, *Medicago sativa* L., located at the University of Arizona-Maricopa Agricultural Center, Pinal County, Arizona. *L. lineolaris* colonies were started from collections made in alfalfa; horseweed, *Conyza canadensis* (L.) Cronquist; redroot pigweed, *Amaranthus retroflexus* L.; giant ragweed, *Ambrosia trifida* L.; and crimson clover, *Trifolium incarnatum* L. from west central Mississippi, southeastern Arkansas, and northeastern Louisiana. Feral individuals, from the same geographic areas and host plants, were added to the colonies several times per year to maintain genetic diversity.

L. hesperus and *L. lineolaris* were placed in separate incubators maintained at $25 \pm 1^\circ\text{C}$ day and $23 \pm 1^\circ\text{C}$ night, $55 \pm 15\%$ RH, with a photoperiod of 14:10 (L:D) h. Both species were reared on green beans; carrots; pink bollworm, *Pectinophora gossypiella* (Saunders) eggs; and a 10% sucrose solution. The green beans and carrots also served as oviposition substrates. Food was changed every other day, and the previously used beans and carrots were placed in 2 by 14-cm-diameter petri dishes that were lined with filter paper and maintained in an incubator until first instars of *Lygus* spp. emerged. Nymphs were placed in 8.5 by 12.5-cm-diameter paper cartons where the center of each lid had been replaced with nylon organdy to allow air circulation. Fresh food was provided every other day until adults emerged. Adults were collected within 24 h of emergence and maintained in paper cartons with fresh diet until they reached the desired age.

Flight Mill Assays. Tethered flight assays of adult *L. hesperus* and *L. lineolaris* were carried out with computer-interfaced flight mills, originally described by Wales et al. (1985) and Barfield et al. (1988) and modified by Naranjo (1990a, b). The insect was tethered to the end of a balanced, lightweight lever that pivoted on a fulcrum, which allowed the insect to fly only in a vertical plane. A flag was attached to the opposite end of the lever and blocked (when insect was resting) or did not block (when insect was flying) an infrared beam that generated an electrical signal that was interpreted by the computer. Activity sensors (infrared emitter-detectors) were shielded so that flight activity could be evaluated during the photophase. When the insect was at rest it was suspended ≈ 2 mm above the floor of the unit. The flight mill system consisted of 24 individual units, which were contained within vented boxes constructed of Plexiglas and wood. Flight mills were located in a room maintained at $25 \pm 1^\circ\text{C}$ day and $23 \pm 1^\circ\text{C}$ night, $48 \pm 2\%$ RH, with a photoperiod of 14:10 (L:D) h. The beginning of photophase in the room corresponded to the beginning of photophase in the environmental chambers where *L. hesperus* and *L. lineolaris* were maintained. Light intensity in the takeoff position was 372.6 ± 15.5 lux during photophase.

Lygus bugs were anesthetized with CO_2 (exposure time < 1 min) and tethered with dental wax by the

pronotum to the end of a quilting thread (0.35 mm diameter), the other end of which was connected to a small copper tube (10 mm by 1 mm i.d.), which fit over the end of the lever described above. Flight activity of *Lygus* bugs was examined over a 23-h period beginning at 1200 hours. For each insect, the computer recorded the clock time, and number and duration of each flight made. Insects that died during the assays were excluded from analyses. Most female bugs were collected at the end of the assay and placed in 70% alcohol for dissection to determine the number of mature chorionated eggs and spermatophores within the seminal depository (Strong et al. 1970). Herein, we used the term "spermatophore" for the paired structures that contained spermatozoa and were found inside the seminal depository.

Vertical Flight Chamber Assays. Untethered flights were monitored in a vertical flight chamber described by Blackmer and Phelan (1991) and Blackmer and Byrne (1993). The chamber had a Philips 400-W mercury-vapor lamp that was suspended above to simulate skylight. It was equipped with a flo-multimeter (Sierra Instruments, Monterey, CA) connected to a strip-chart recorder (model BD 11 E, Kipp & Zonen Inc., Delft, The Netherlands), which enabled us to measure air speed and provided an indirect record of the insects' rate of climb. At the onset of a test, vials containing five bugs were placed on a platform 11 cm above the chamber floor. At this platform, light intensity measured 910 ± 1.7 lux. Insects were preconditioned to the overhead lamp and temperature for 30 min before a vial was opened. *Lygus* bugs were flown 10–15 cm below the light window by manually adjusting the airflow into the system. Insects were given 5 min to respond to the overhead light. Percentage of bugs taking off, percentage exhibiting phototactic orientation, and flight duration of each flight were noted. To be considered a phototactic flight, the bug had to maintain its flight to the overhead light for > 30 s. If a bug landed during the bioassay, it was given 2 min to reinitiate its' flight. If it failed to reinitiate flight within this period, it was removed from the chamber.

For studies on the effect of light intensity on takeoff activity, the chamber was equipped with a tungsten/halogen 500-W light (Regents Lighting Corp., Burlington, NC). This type of light enabled us to adjust intensity by means of a dimmer switch. Light levels were measured with a light meter (ExTech Instruments model 401025, Zefon International, St. Petersburg, FL), and were set at 0, 3, 10, 20, 50, 100, 200, and 360 lux. At the beginning of each trial, vials containing five, 1–2-wk-old *L. hesperus* or *L. lineolaris* were placed on the chamber floor and held in darkness under black fabric. Light levels were presented in increasing intensities, and on a given day, all intensities were tested beginning shortly after the start of the photoperiod until approximately mid-day. Five to 11 groups of individuals were tested for each species and light intensity. Night-vision goggles (model M-802, Litton, Tempe, AZ) were used to measure takeoff activity at light levels < 10 lux. During all untethered flight trials, the flight chamber was maintained at $27 \pm 1^\circ\text{C}$ and

48 ± 2% RH, and tests were conducted from 0600 to 2000 hours.

Statistical Analyses. Flight propensity and duration were determined in relation to species, age, and sex for both flight assay systems. Time-of-day experiments were limited to photophase in the vertical flight chamber. Four age groups, roughly representing prereproductive (1–6 d old) and early through late reproductive (7–13, 14–21, and >21 d old) were examined. For each species, sex, and age group, between 82 and 250 bugs were assayed on the flight mills, and between 13 and 112 were assayed in the vertical flight chamber. After correcting for continuity with Yates' correction factor (Zar 1984), χ^2 tests were used to test for differences in the percentage of male and female *L. hesperus* and *L. lineolaris* flying, as well as differences in daily flight periodicity (trivial and sustained for tethered flight) between the species and sexes. Tethered flights were categorized and analyzed according to individual duration, with trivial flights lasting <5 min and sustained flights lasting >5 min. This division was somewhat arbitrary, but was based on the frequency of individual flights of varying duration.

Three-way analysis of variance (ANOVA) was used to examine the effects of species, age, and sex on flight activity. The number of flights taken over the 23-h period (for tethered flights) and flight duration of trivial and sustained flights were compared. Where significant age differences were indicated, means were separated by Tukey's honestly significant difference (HSD) test. Flight data were log transformed or ranked before analyses when they failed to meet the assumptions of normality and equal variance.

To determine whether the number of eggs and/or number of spermatophores differed between fliers and nonfliers for *L. hesperus* and *L. lineolaris*, we conducted *t*-tests. Two-way ANOVAs on ranked data were used to determine whether egg load and number of spermatophores varied between the species and across age groups. For fliers only, Spearman rank order correlation analyses were used to determine whether flight parameters were correlated with changes in egg load and/or number of spermatophores. Correlation coefficients were generated for the main effects of species and age. Due to low sample sizes and similarities in flight capacity for 7–21-d-old bugs, we combined these age groups before correlation analyses were run.

For vertical flight chamber data, χ^2 tests were used to test for differences in the percentage of male and female *L. hesperus* and *L. lineolaris* flying and exhibiting phototactic orientation, as well as differences in flight periodicity during the day. Three-way ANOVAs on ranked data were used to examine the effect of species, age, and sex on flight propensity and rates of climb; however, because of the small number of sustained flights, all flight data were combined regardless of duration. The effect of light intensity on takeoff activity for each species was examined by fitting the model $Y = A(1 - e^{-bx})$, where Y is the mean proportion taking off, x is light intensity, A is the asymptote, and b measures the rate of change in proportional

takeoff to light intensity. The regression models were fit using the Marquardt–Levenberg method in SigmaPlot V. eight (SPSS Inc., Chicago, IL). The parameters of the model between species were compared using *t*-tests.

Results

Tethered Flight Assays. More than 1,000 *L. hesperus* and 1,400 *L. lineolaris* were tethered; however, the number of bugs that flew during the assays varied from 29 to 54 for each age group, species, and sex combination. Assay mortality was low (<10%) for *L. hesperus* females through >21 d old but reached ≈24% for *L. lineolaris* females >21 d old. Mortality was always higher for males and exceeded 35% in individuals >21 d old. A large percentage of the bugs (60–86%) was categorized as nonfliers, with the majority occurring in the 1–6-d-old bugs. Male *L. lineolaris* that were >21 d old also had a large percentage of nonfliers (86%). For bugs that flew, 60–97% engaged in trivial flights depending on species, sex, or age. The largest percentage of sustained flights occurred in 14–21-d-old individuals, regardless of species or sex.

Considering all flights, regardless of duration, there were significant differences due to species, sex, and age in the percentage of individuals that flew. A larger percentage of *L. hesperus* engaged in flight compared with *L. lineolaris* (35 versus 29%; $\chi^2 = 8.60$, $df = 1$, $P = 0.003$), females flew more often than males (35 versus 28%; $\chi^2 = 9.8$, $df = 1$, $P = 0.002$), and large differences existed due to age category ($\chi^2 = 32.5$, $df = 3$, $P = 0.001$). The smallest percentage (24%) of fliers occurred in the 1–6-d-old bugs, and the largest percentage (38%) occurred in the 14–21-d-old bugs.

When combined over age groups, individual flight frequency distributions were similar, regardless of sex or species. A single flight was taken by ≈14% of the bugs; however, there were some individuals of both species and sex that took >200 flights during the assays (Fig. 1). There was considerable variation in the frequency distributions for individual mean and cumulative flight duration relative to species and sex. Females generally engaged in more flights of longer mean duration than males. For cumulative flight duration, the longest flights were exhibited by two male *L. hesperus* that flew in excess of 700 min during the assays; roughly one-half the total time possible. Females, however, engaged in more flights of longer cumulative duration than males.

For subsequent analyses, an individual's flight performance was categorized as trivial if it never initiated a single flight >5 min in duration, or sustained if it took at least one flight >5 min in duration during the assay. For individuals that exhibited both trivial and sustained flights, means and cumulative values were calculated for each category.

Trivial Flights. For individuals that engaged in trivial flights, there were no significant interactions relative to species, sex, or age groups for any of the trivial flight parameters. For main effects, there were no significant differences between the species for num-

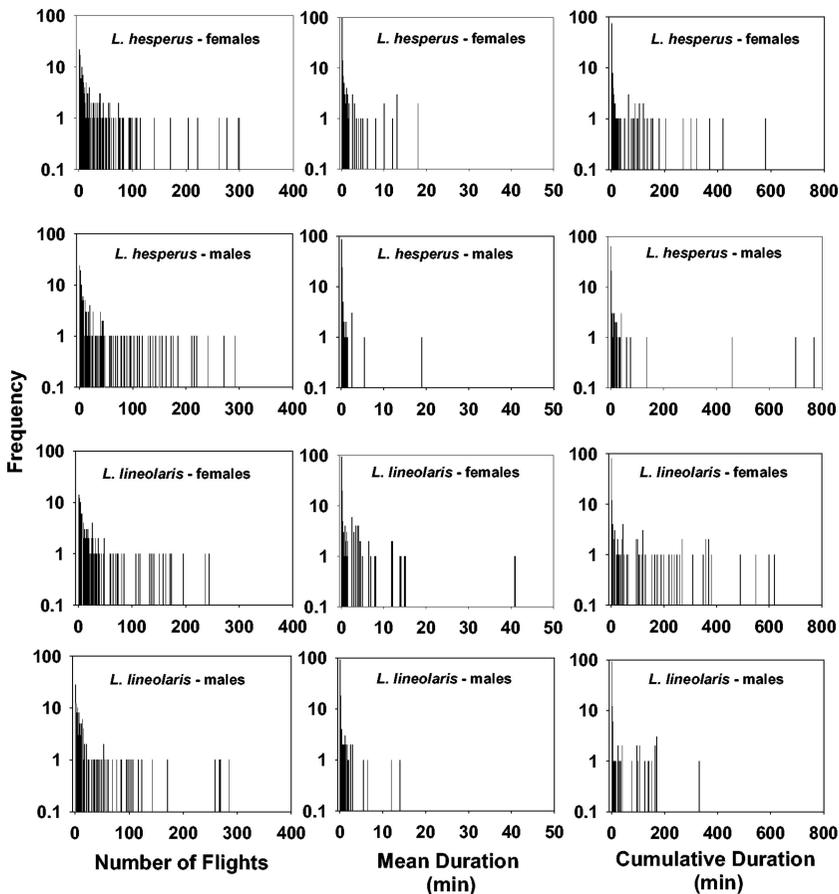


Fig. 1. Frequency distributions for number of flights, and mean and cumulative duration (minutes) of flights made by male and female *L. hesperus* and *L. lineolaris* during 23-h tethered flight assays.

ber of flights taken, for mean flight duration, or for cumulative flight duration (Fig. 2; $P > 0.05$). There also were no significant differences between the sexes in terms of number of trivial flights and mean flight duration, but cumulative trivial flight duration differed, with females exhibiting longer cumulative flights than males (Fig. 2; $F = 3.84$; $df = 1, 611$; $P = 0.05$). Relative to age, there were significant differences for all three trivial flight parameters (Fig. 2). Individuals that were >21 d old took significantly more flights than 1–6-d-old individuals ($F = 3.99$; $df = 3, 611$; $P = 0.008$). For mean trivial flight duration, 14–21-d-old individuals had significantly longer flights than 7–13-d-old and >21 -d-old individuals ($F = 3.60$; $df = 3, 611$; $P = 0.013$). For cumulative flight duration, 14–21-d-old individuals engaged in significantly longer flights than 7–13-d-old individuals ($F = 2.89$; $df = 3, 611$; $P = 0.035$).

Sustained Flights. For individuals that engaged in sustained flights, there were no significant interactions for any of the parameters relative to species, sex, or age groups. For main effects, *L. lineolaris* engaged in more flights than *L. hesperus* (Fig. 3; $F = 6.64$; $df = 1, 115$; $P = 0.011$), and these flights were of longer cumulative duration ($F = 4.01$; $df = 1, 115$; $P = 0.048$). However,

mean flight duration was not significantly different between the species ($P > 0.05$). Females took over twice as many sustained flights as males (Fig. 3; $F = 9.48$; $df = 1, 115$; $P = 0.003$), and these flights were of longer cumulative duration ($F = 7.03$; $df = 1, 115$; $P = 0.009$). Mean flight durations were not significantly different between the sexes ($P > 0.05$). Individuals that were 14–21-d old took more flights than 1–6-d-old individuals (Fig. 3; $F = 2.79$; $df = 3, 115$; $P = 0.044$); however, there were no significant differences among age categories for mean or cumulative flight durations ($P > 0.05$).

Flight Periodicity. *L. hesperus* and *L. lineolaris* engaged in trivial flights throughout the day and night (Fig. 4). Frequency of flight activity varied similarly for male and female *L. hesperus*, with predominantly diurnal to crepuscular activity. Most trivial flights occurred between 1400 and 2400 hours and then again beginning at approximately lights-on, or 0600–1100 hours. For both sexes, flight activity was reduced from 0100 to 0500 hours. Despite these similarities, trivial flight periodicities differed between sexes ($\chi^2 = 283.5$, $df = 23$, $P = 0.001$), with the frequency of male flights being noticeably reduced from 1600 to 2400 hours compared with female flights.

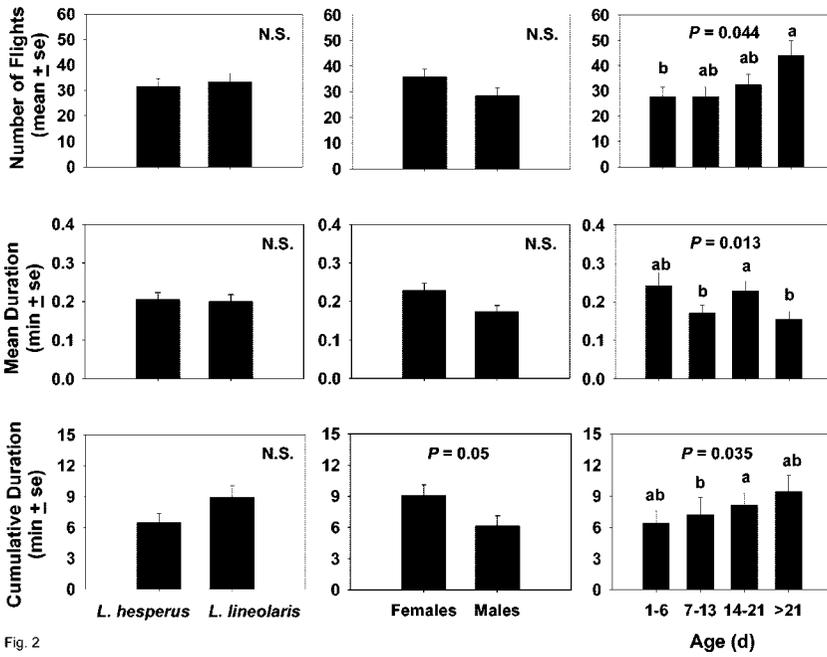


Fig. 2

Fig. 2. Comparisons between *L. hesperus* and *L. lineolaris*, females and males, and among age groups for number of trivial flights, and mean and cumulative trivial flight duration during 23-h tethered flight assays. ANOVAs based on ranked data, but untransformed means ± SE are illustrated. Age group means with the same letter are not significantly different at $P < 0.05$ (Tukey's HSD).

Female *L. lineolaris* followed a similar pattern, with the greatest trivial flight activity occurring from 1400 to 2200 hours and then again from 0500 to 1000 hours, separated by a decrease in flight activity from 2300 to

0400 hours (Fig. 4). Male *L. lineolaris*, in contrast, did not exhibit any distinct patterns in flight frequency, and differences in pattern of flight activity between the sexes were significant ($\chi^2 = 272.0$, $df = 23$, $P <$

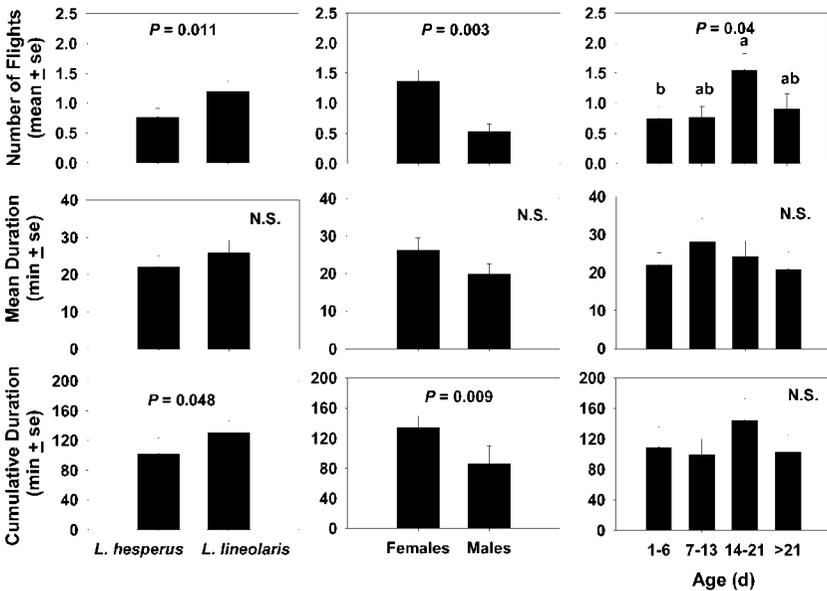


Fig. 3. Comparisons between *L. hesperus* and *L. lineolaris*, females and males, and among age groups for number of sustained flights, and mean and cumulative sustained flight duration during 23-h tethered flight assays. ANOVAs based on ranked data, but untransformed means ± SE are illustrated. Age group means with the same letter are not significantly different at $P < 0.05$ (Tukey's HSD).

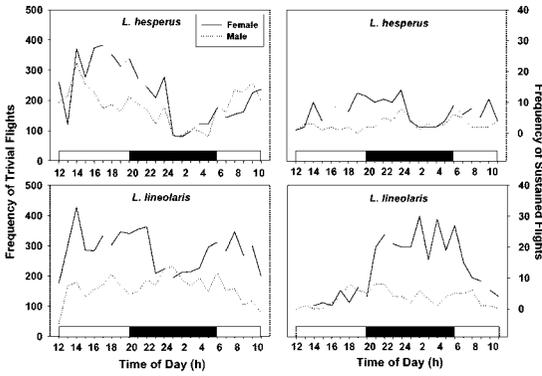


Fig. 4. Frequency of trivial and sustained flights relative to time of day (hours) for female and male *L. hesperus* and *L. lineolaris*. White bars at base of graph represent photophase, whereas dark bars represent scotophase. Species are presented separately for illustration purposes but were analyzed in side-by-side comparisons by χ^2 tests.

0.001). Comparisons between species, by sex, identified significantly different periodicities for females ($\chi^2 = 448.4$, $df = 23$, $P < 0.001$) and males ($\chi^2 = 549.6$, $df = 23$, $P < 0.001$).

Flight periodicities for sustained flights showed some similarities to trivial flights, as well as some unique features. The periodicity of sustained flights for *L. hesperus* females was almost identical to the periodicity of their trivial flights. Most activity occurred from 1400 to 2400 hours and again from 0600 to 1000 hours, with a depression in activity from 0100 to 0500 hours (Fig. 4). Periodicity of sustained flights for males was more narrowly defined than was the case for their trivial flights. Peaks in activity occurred from 2200 to 0100 hours and again from 0600 to 0700 hours, fitting a nocturnal to crepuscular rather than a diurnal periodicity. For sustained flights, male and female *L. hesperus* did not differ in periodicities ($P = 0.28$), most likely due to the relatively low number of sustained flights.

The periodicity of sustained flights for female *L. lineolaris* contrasted sharply with the periodicity of their trivial flights. A marked increase in flight activity occurred at 2000 hours, followed by a sharp decrease at 0600 hours, indicating that their sustained flights were made almost exclusively at night (Fig. 4). Males, however, showed a pattern that was similar to the periodicity of trivial flights where no distinct period of activity was evident. Differences in sustained flight periodicities between male and female *L. lineolaris* were significant ($\chi^2 = 53.1$, $df = 23$, $P < 0.001$). Comparisons between species, by sex, identified significantly different periodicities of sustained flights for females ($\chi^2 = 102.7$, $df = 23$, $P < 0.001$) and males ($\chi^2 = 35.3$, $df = 23$, $P < 0.05$).

Flight Behavior and Reproductive and Mating Status. For comparisons of number of eggs and spermatophores, we excluded <3-d-old bugs from the data set. This was justified because mating does not occur until after 3 d of age (J.L.B., unpublished data), eggs were only observed in 4 d or older individuals, and for most

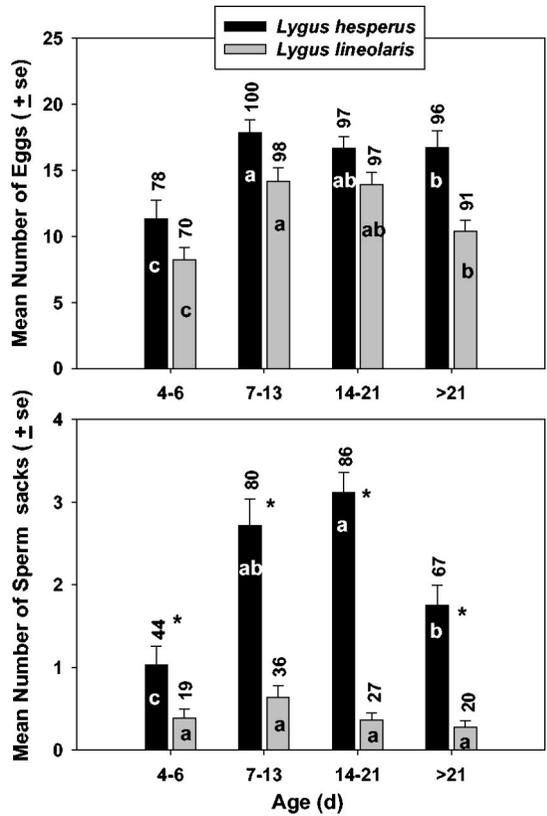


Fig. 5. Mean number of mature eggs and spermatophores for *L. hesperus* and *L. lineolaris* in relation to age. ANOVAs based on ranked data, but untransformed means \pm SE are illustrated. Means with the same letter for a given species are not significantly different at $P < 0.05$ (Tukey's HSD). Numbers above bars represent percentage of individuals that had at least one or more eggs or spermatophores. * indicates when species were different within an age group.

insects there is a teneral period after emergence when flight is not possible. The remaining 497 females were dissected after flight assays. Of these, 147 (30%) were fliers and 350 (70%) were nonfliers. There were no significant differences between fliers and nonfliers in egg load ($t = 1.64$, $df = 495$, $P = 0.10$) or spermatophores ($t = 1.09$, $df = 495$, $P = 0.28$). Consequently, we pooled fliers and nonfliers before analyzing for species and age effects.

When combined over all age groups, *L. hesperus* had higher egg loads than *L. lineolaris* (15.6 ± 0.6 versus 11.5 ± 0.5 eggs, respectively; $F = 35.33$; $df = 1, 489$; $P < 0.001$), and when combined over species, egg load varied significantly with age ($F = 14.47$; $df = 3, 489$; $P < 0.001$). For both species, 4–6-d-old bugs had the fewest eggs; however, 70–78% of these bugs had one or more eggs (Fig. 5). More than 90% of the older bugs had one or more eggs, with 7–13-d-old bugs having significantly more eggs than bugs that were 4–6 and >21 d old.

L. hesperus also had a higher number of spermatophores than *L. lineolaris* (2.16 ± 0.10 versus $0.41 \pm$

Table 1. Untethered flight statistics for male and female *L. hesperus* and *L. lineolaris* relative to sex and age

Species, sex, age category	No. assayed	% Takeoff	% Phototactic	% Sustained flights
<i>L. hesperus</i> females				
1-6 d	13	54	14	0
7-13 d	22	68	47	0
14-21 d	92	55	47	12
>21 d	13	77	20	0
<i>L. hesperus</i> males				
1-6 d	16	62	20	0
7-13 d	30	63	47	0
14-21 d	85	73	58	6
>21 d	29	76	64	0
<i>L. lineolaris</i> females				
1-6 d	20	65	15	0
7-13 d	99	68	40	0
14-21 d	44	70	42	0
>21 d	13	54	29	0
<i>L. lineolaris</i> males				
1-6 d	14	64	22	0
7-13 d	112	75	50	12
14-21 d	39	64	52	31
>21 d	14	79	73	12

0.08, respectively; $F = 180.88$; $df = 1, 489$; $P < 0.001$), and the number varied with age ($F = 12.99$; $df = 3, 489$; $P < 0.001$). For *L. hesperus*, there were more spermatophores in 14-21-d-old individuals than in 4-6- and >21-d-old individuals. Also, 86% of the individuals in this age group had at least one spermatophore, whereas only 44-67% of the younger and older individuals had spermatophores (Fig. 5). For *L. lineolaris*, there were no differences in the number of spermatophores relative to age, and the percentage of individuals that had spermatophores was relatively low (19-36%). *L. hesperus* had a higher number of spermatophores than *L. lineolaris* at all age categories ($F = 7.28$; $df = 3, 489$; $P < 0.001$).

We found no significant associations between egg load or number of spermatophores and any of the flight parameters for *L. lineolaris* ($P > 0.05$). Similarly, there were no significant associations between egg load and flight parameters for *L. hesperus* ($P > 0.05$); however, the number of spermatophores was positively associated with mean flight duration (both trivial [$r = 0.34$, $n = 57$, $P = 0.01$] and sustained [$r = 0.59$, $n = 13$, $P = 0.03$]), and cumulative sustained flight duration ($r = 0.61$, $n = 13$, $P = 0.02$). In relation to age, egg load was correlated with number of sustained flights ($r = 0.66$, $n = 12$, $P = 0.02$), and egg load and number of spermatophores were correlated with cumulative duration of sustained flights in 4-6-d-old individuals ($r = 0.73$, $n = 12$, $P = 0.006$ and $r = 0.62$, $n = 12$, $P = 0.03$, respectively). In 7-21-d-old individuals, number of spermatophores and number of trivial flights were negatively correlated ($r = -0.25$, $n = 73$, $P = 0.03$), whereas in individuals >21 d old, egg load was negatively correlated with number of trivial flights ($r = -0.31$, $n = 47$, $P = 0.04$) and cumulative duration of trivial flights ($r = -0.32$, $n = 47$, $P = 0.03$).

Untethered Flight Assays. A total of 300 *L. hesperus* and 355 *L. lineolaris* were given the opportunity to initiate flight in the vertical flight chamber; the actual number that flew varied from 7 to 84 for each age group, species, and sex combination. The percentage

taking off in flight was always >50%, tended to increase with age, and was slightly higher in males than females. A better measure of an insect's tendency to fly for any length of time in the vertical flight chamber is the percentage that engages in a phototactic response (i.e., a prolonged flight where the insect remains within the light window). In terms of this response, females were more likely to engage in phototactic orientation from 7 to 21 d of age, whereas in males the response gradually increased with age (Table 1). For bugs that exhibited phototactic responses, their flights could best be categorized as trivial in nearly all cases. Longer flights for *L. hesperus* only occurred in 14-21-d-old individuals and *L. lineolaris* females never exhibited sustained flights. Male *L. lineolaris*, however, engaged in sustained flights in all age groups except the youngest.

When all flights were considered, regardless of whether they were phototactic or not, there were no significant differences due to species, or age in the percentage of individuals that flew ($P > 0.05$). However, males were more likely to initiate flight than females (71 versus 64%; $\chi^2 = 4.17$, $df = 1$, $P = 0.041$). In terms of their phototactic responses, there were no significant differences due to species ($P > 0.05$); however, males engaged in phototactic orientation more often than females (52 versus 39%; $\chi^2 = 7.25$, $df = 1$, $P = 0.007$), and there were differences among the four age groups ($\chi^2 = 14.7$, $df = 3$, $P = 0.002$). The 1-6-d-old individuals rarely exhibited a phototactic response (18%).

When combined over age groups, individual flight frequency distributions were similar, with the exception of one male *L. hesperus* (Fig. 6). For 69% of the individuals flown, only one flight was recorded, although this percentage probably would have been lower had the criterion for reinitiating flight been >2 min. Several individuals took >15 flights during the assays, and one male took 61 flights. For individual mean and cumulative flight durations, there was substantial variation in frequency distributions, relative to

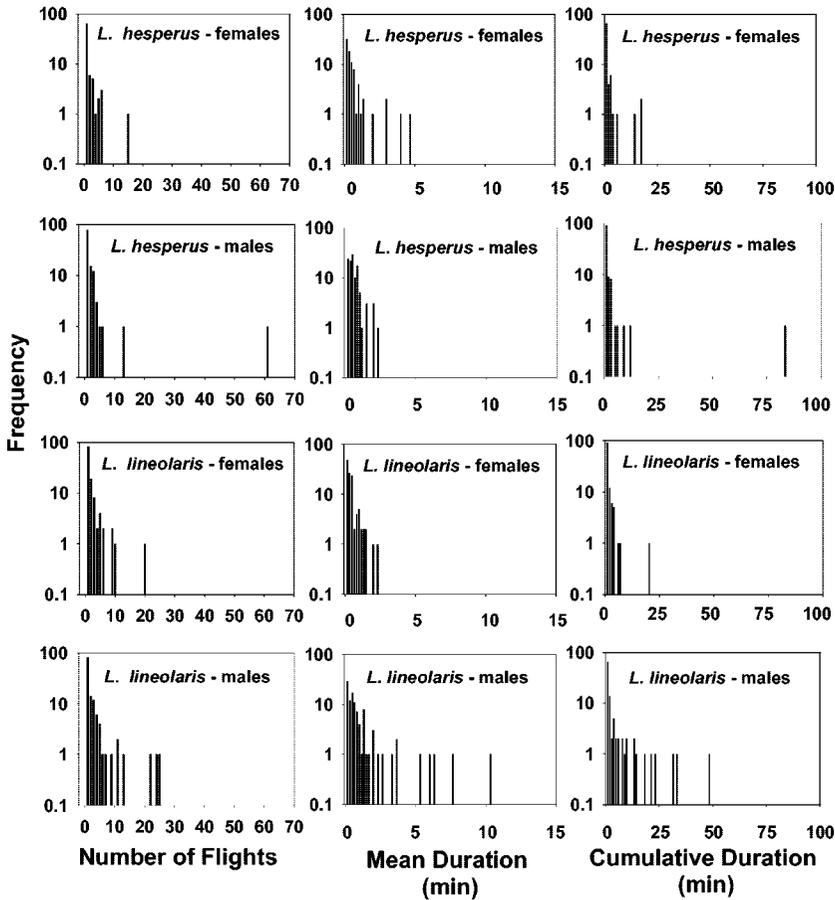


Fig. 6. Frequency distributions for number of flights, and mean and cumulative duration (minutes) of flights made by male and female *L. hesperus* and *L. lineolaris* during untethered flight assays in a vertical flight chamber.

species and sex (Fig. 6). Female *L. hesperus* and male *L. lineolaris*, in general, engaged in flights of longer mean duration than male *L. hesperus* and female *L. lineolaris*. In terms of cumulative flight duration, males exhibited the longest flights.

For individuals that engaged in flights, there were no significant interactions relative to species, sex, and age for any of the flight parameters. For main effects, there were no significant differences between the species or among the various age groups for number of flights taken, for mean flight duration or for cumulative flight duration ($P > 0.05$). There also were no differences between the sexes in terms of the number of flights taken. There was, however, a difference between the sexes in terms of mean and cumulative flight duration, with males engaging in longer flights than females ($F = 9.34$; $df = 1, 427$; $P < 0.004$ and $F = 6.67$; $df = 1, 427$; $P < 0.01$, respectively).

L. hesperus and *L. lineolaris* engaged in flights throughout the day in the flight chamber. No strong patterns of activity were evident for females. For male *L. hesperus*, the number of flights peaked at 1000 hours, but this was due to one male that took 61 flights. For male *L. lineolaris*, flight activity peaked from 1,000 to

1,500 hours. Despite these fairly weak patterns, there were significant differences in activity between male and female *L. hesperus*, even when the male outlier was eliminated ($\chi^2 = 57.9$, $df = 14$, $P = 0.001$). Differences in activity were evident for male and female *L. lineolaris* ($\chi^2 = 69.9$, $df = 14$, $P = 0.001$). Differences also existed between the females ($\chi^2 = 33.3$, $df = 14$, $P = 0.001$) and males ($\chi^2 = 59.8$, $df = 14$, $P = 0.001$ without outlier).

Untethered flights, although of shorter duration, displayed all the characteristics that were seen for tethered flights. There were numerous takeoffs, in which short flights were separated by relatively short periods of inactivity (Fig. 7A). In a few cases, a sustained flight occurred that was followed by short flights separated by short periods of inactivity (Fig. 7B). The general characteristics of these flights resembled those of other insects that have been flown in the flight chamber (Blackmer and Phelan 1991, Blackmer and Byrne 1993, Blackmer and Cross 2001), where rates of climb gradually declined over time. Rates of climb did not differ among age groups or between species ($P > 0.05$), but males had higher rates of climb than females ($F = 5.74$; $df = 1, 282$; $P = 0.017$). Both

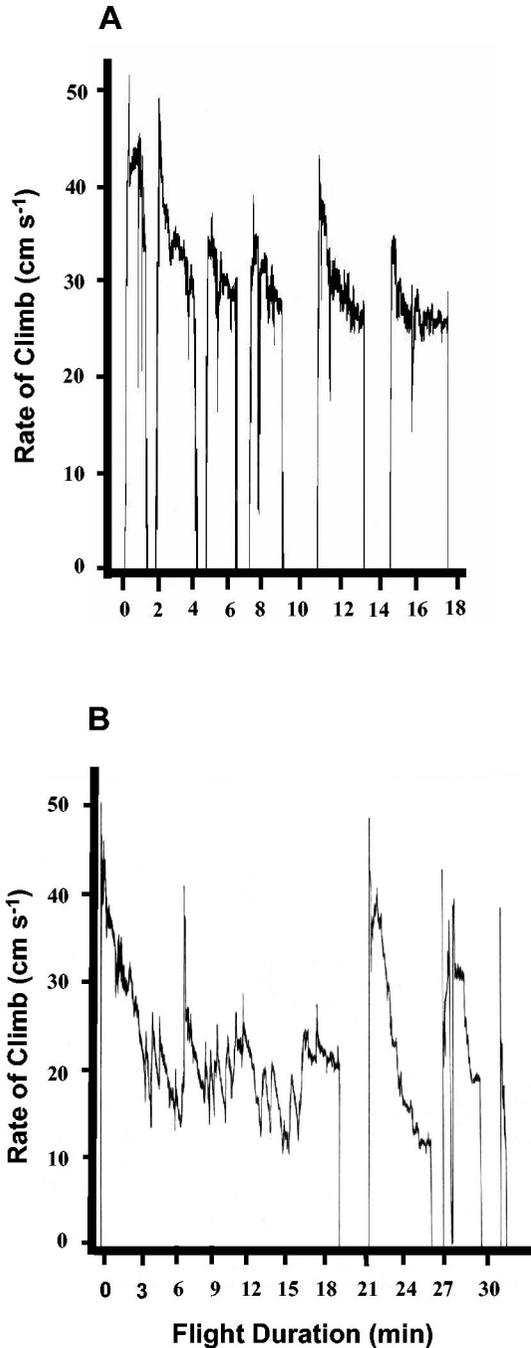


Fig. 7. Representative strip-chart recordings of (A) trivial flights made by a female *L. hesperus* and (B) sustained flights made by a male *L. lineolaris* in a vertical flight chamber.

species and sexes demonstrated a capacity for strong, self-directed flight, with rates of climb reaching $\approx 50 \text{ cm s}^{-1}$.

Because we observed a predominance of short-duration flights in the vertical flight chamber, we examined whether light intensity was influencing flight

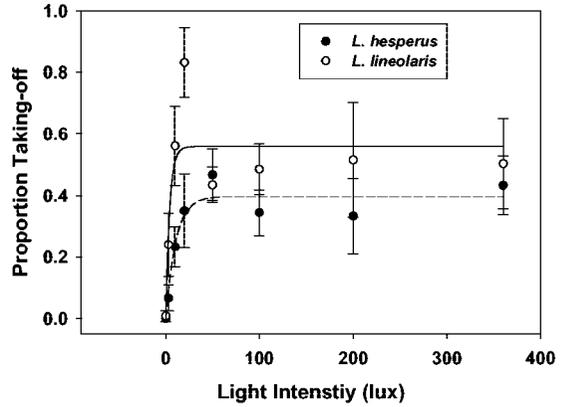


Fig. 8. Relationship between light intensity (x) and take-off (y) response by *L. hesperus* ($y = 0.40 * [1 - e^{-0.09x}]$, $r^2 = 0.94$, $n = 8$) and *L. lineolaris* ($y = 0.56 * [1 - e^{-0.25x}]$, $r^2 = 0.73$, $n = 8$) in a vertical flight chamber.

activity. A few *L. hesperus* initiated flights at 3 lux, but flight activity in response to the overhead light leveled off at ≈ 0.40 beginning at 50 lux (Fig. 8). A few *L. lineolaris* initiated flights at 0 lux, and this was followed by a marked increase in take off that leveled off at 0.56 beginning at a light level of 20 lux. The asymptotes of the regression models differed significantly ($t = 2.53$, $df = 14$) between species, indicating that takeoff rates were higher for *L. lineolaris*, regardless of light intensity. The rate of change in flight activity relative to light intensity was similar between the species ($P > 0.05$).

Discussion

Numerous biotic and abiotic factors probably influence the timing and extent of dispersal by *L. hesperus* and *L. lineolaris*. Both species are highly polyphagous but have different distributions in North America. Their different distributions may reflect a divergence in dispersal strategies, the distribution of their various hosts, or the interaction between dispersal and host plants may have shaped the extent of their current geographic ranges.

Few studies have examined factors that influence the flight behavior of *Lygus* bugs. Stewart and Gaylor (1994) presented a detailed study of the tethered flight of *L. lineolaris*. Their study differed from ours in that their flight mills allowed horizontal flight, whereas ours permitted vertical flight, age groupings were slightly different, and data were collected for 12 rather than 23 h. Our designation of fliers versus non-fliers also differed, in that we considered all individuals that flew for any length of time to be fliers, whereas they considered only those individuals that had cumulative flight durations >10 min to be fliers. Despite these differences in methodology, many of our findings were similar for *L. lineolaris*. A large percentage of bugs was classified as nonfliers in both studies, very few individuals initiated flight before 4 d of age, the number of flights made during the assays

varied significantly with age, reproductive females had longer cumulative flights than prereproductive females, and more flights of longer cumulative duration were registered for female than for male *L. lineolaris*. Approximately 42% of their females and 16% of their males engaged in cumulative flights >10 min. If we use their criterion, and exclude *L. lineolaris* that flew for <10 s, ≈46% of our females and 26% of our males engaged in cumulative flights >10 min.

Dissimilar to our tethered flight study, Stewart and Gaylor (1994) reported a decline in flight activity for bugs >14 d old; however, this was their oldest age category, and they never mentioned the range in age for this group. Our 14–21-d-old bugs engaged in more sustained flights than any other age group; however, individuals that were >21 d old (maximum of 38 d) were less likely to engage in sustained flights.

No such studies had previously been conducted for *L. hesperus*, which in our tethered flight study exhibited many similarities with *L. lineolaris*. Females flew more often than males, but males and females for both species were capable of flying for extended periods, with some individuals taking >200 flights during the assays. In addition, older reproductive individuals (14–21 d old) had flights of longer duration than did the youngest and oldest individuals, regardless of sex or species. The most important difference between the species, in terms of flight capacity, was that *L. lineolaris* engaged in more sustained flights than *L. hesperus*, which translated into longer cumulative sustained flight durations. The higher degree of polyphagy for *L. lineolaris* combined with more sustained flights could possibly explain its more cosmopolitan distribution. Detailed studies of host distributions, seasonal availability, and preferences would need to be conducted to better clarify this possibility.

Large differences between the species and sexes were observed when the periodicities of trivial and sustained flights were analyzed. Both sexes of *L. hesperus* exhibited mostly diurnal to crepuscular trivial flights; however, for sustained flight, the response more closely fit a crepuscular periodicity, especially for males. Frequency of trivial flights for female *L. lineolaris* was similar to the frequency exhibited by male and female *L. hesperus*; male *L. lineolaris* did not demonstrate a distinct periodicity. The frequency of sustained flights for *L. lineolaris* females, however, was distinct, in that nearly all sustained flights occurred at night.

Periodicity of flight activity for these two species has been examined under field conditions on a very limited basis. Butler (1972) trapped *L. hesperus* during the day and night over a period of 3 d in central Arizona and was able to identify a crepuscular periodicity that peaked 1 h after sunset and 1 h before sunrise. Very low levels of activity occurred at other times of the day, but collections were not attempted when wind speeds were high, and during these collections, daytime temperatures reached 43°C, which may have suppressed flight activity. Mueller and Stern (1973) examined the periodicity of flight activity of *L. hesperus* in the San Joaquin Valley, CA, by using two

types of traps (sticky yellow poles and aerial rotating nets). Similar numbers of males and females were trapped, and the majority of bugs were trapped within the first 2 h after sunset. No early morning peak in activity was detected in CA, and with the yellow pole traps, a small number of individuals were collected throughout the day and evening. These individuals were probably engaging in trivial, host-finding behavior as yellow was previously found to be attractive to *L. hesperus* (Landis and Fox 1972). The extended flight periodicity in the laboratory, relative to these two field studies, is probably a result of the rather uniform conditions. Variations in wind speed, temperature, and other less understood abiotic factors would play a role in the timing of takeoffs and flight duration.

For *L. lineolaris*, several individuals were collected 5 km off shore at night in June and July with light traps (MacCreary 1965). These were clearly sustained flights, because there were no other land masses in the area. In terms of their daily flight activity, Rancourt et al. (2000) showed that most adults were captured at mid-day (1000–1400 hours) on white-sticky traps, which are thought to mimic apple flower bud and blossoms in their reflectance pattern (Prokopy et al. 1979). Apparently, male and female *L. lineolaris* are differentially attracted to these traps, or males are engaging in flight activity more often, as indicated by a biased trap ratio in several studies where the background sex ratios were close to parity (Prokopy et al. 1979, Boivin et al. 1982, Boivin and Stewart 1984). Although it is difficult to make comparisons between our laboratory assays and these field studies, it is apparent that both in the laboratory and field, male and female *L. lineolaris* respond differently to their environment, whereas male and female *L. hesperus* are more similar in their flight responses. These differences in periodicities have probably evolved in response to environmental factors. By restricting flights to certain times of the day or night, desiccation, predation, or the harmful effects of radiation might be reduced. These differences in periodicity should be considered when developing and interpreting management and monitoring plans based on adult trapping. Trap efficacy would differ between the sexes and species because of their different behavioral responses.

Although environmental parameters obviously play a large role in the timing of flight, physiological constraints also have an impact. One of the factors that Stewart and Gaylor (1994) identified as influencing flight behavior in *L. lineolaris* was its reproductive status; mating status apparently had no effect in their study. They found that reproductive females made most of the long-duration flights, and in fact had cumulative flight durations seven times greater than those without eggs. These results support the findings of Stewart and Gaylor (1991) that young, reproductive females of this species were most inclined to colonize host patches. We found that egg load varied significantly with age for both species, where it was higher in 7–13-d-old individuals than in 4–6- and >21-d-old individuals, but it was not different from egg loads in

14–21-d-old individuals. In this respect, egg load was highest for both species when most of the longest flights were occurring; however, none of the correlation coefficients were significant for *L. lineolaris* egg load or number of spermatophores and flight activity, or for egg load and flight activity for *L. hesperus*. The number of spermatophores per female, however, was positively correlated with mean trivial, mean sustained, and cumulative flight duration for *L. hesperus*. It is hard to imagine how an increasing number of spermatophores, from what are obviously multiple matings for *L. hesperus*, could contribute to enhanced flight potential. It seems more likely that the co-occurrence of increases in flight activity and mating activity are related to age in a similar manner. After mating, however, this insect does engage in cyclical nonreceptivity that lasts for ≈ 6 d (Strong et al. 1970), and during this period, females might be more likely to engage in longer flights. A more detailed study of mating behavior and sperm load relative to flight behavior might resolve this point.

When we examined the effect of age, regardless of species, there were significant positive correlations between egg load and number and cumulative duration of sustained flights in 4–6-d-old individuals. In terms of the number of spermatophores, there was also a positive correlation with cumulative duration of sustained flights. In these younger bugs, the reproductive systems, as well as the flight apparatus are in a process of maturation. Physiological changes such as hardening and darkening of the cuticle, development of the flight musculature and mitochondria, maturation of enzymes and substrates needed for flight, and maturation of the reproductive system are occurring simultaneously (Johnson 1969, Blackmer et al. 1995). Given these facts, it is not surprising that we see a positive association between egg load, spermatophores, and sustained flight parameters; however, it is unlikely that increasing egg load and number of spermatophores are directly responsible for increases in the number and cumulative duration of sustained flights. The lack of association between the number of mature eggs and spermatophores for any of the flight parameters in 7–21-d-old individuals, when the number of mature eggs and spermatophores are at their maximum, provides further support that neither of these reproductive measures is directly influencing flight capacity. In older bugs (>21 d), there was an apparent decline in egg load, although the number and cumulative duration of trivial flights remained high, which resulted in a negative correlation coefficient. For many insects, long-duration flights occur before eggs mature, a phenomenon that has been termed the oogenesis-flight syndrome (Johnson 1969, Rankin et al. 1986), but we agree with Stewart and Gaylor (1994), in that *L. lineolaris*, and now *L. hesperus*, do not fit this pattern. Rather, they are well-adapted colonizers that are capable of flying with a full complement of eggs, allowing them to readily exploit new habitats.

An additional goal of our study was to compare and contrast the flight behavior of tethered versus untethered bugs. Both systems have been used extensively to

quantify flight potential in insects, although never in a comparative manner. Kennedy and Booth (1963) believed that the use of tethered systems could distort our understanding of flight owing to the abnormal sensory inputs that are received by the tethered insect. Kutsch and Stevenson (1981) found that tethered locusts produced a lift which compensated for only 70% of their body weight. In addition, wing beat frequency was always significantly higher (except in the first cycle) for untethered versus tethered locusts, and a discrepancy of $\approx 20\%$ was evident regardless of age. Such differences in wing beat frequency could and probably would influence the consumption of flight fuels, as suggested by Kammer and Heinrich (1978). Despite potential shortcomings, the tethered flight system offers a fairly easy way to gather a great deal of information on flight propensity over long periods of time and during the day and night. Such information is either impossible or very difficult to come by with the vertical flight chamber, which is limited in that only one insect can be flown at a time. In addition, our current flight chamber only allows us to interpret flight behavior during photophase, which proved to be a major shortcoming in the current study, as many of our long-duration flights occurred at night.

In spite of the differences and difficulties with each of the systems, we obtained similar results in several respects. With both tethered and untethered flights, the majority of long-duration flights occurred from 14 to 21 d of age, most flights were <5 min in duration, and males in both species and female *L. hesperus* exhibited sustained flights. Furthermore, flight attributes were similar with both systems. Most individuals that took off exhibited numerous short-duration flights that were separated by brief periods of inactivity; however, with both systems, there were also individuals that engaged in sustained long-duration flights. Two of the most important differences between the systems had to do with flight duration and periodicity of flight activity. Both of these differences are because it was possible to collect data from a single individual for much longer periods, and during the night, with the flight mills. With both systems, there were significant differences in flight capacity between the sexes; however, with tethered flight, females engaged in longer flights, whereas for untethered flights, males engaged in longer flights. This difference also may be because we were not able to monitor the long-distance flights at night in the vertical flight chamber. Each system provided us with useful and unique information on these two species, which together, have provided us with a comprehensive picture of many of the factors that influence the flight propensity and duration of *L. hesperus* and *L. lineolaris*.

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