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Effects of Starvation, Age, and Mating Status on Flight Capacity of Laboratory-Reared Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)

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Abstract

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive species to North America and has spread throughout most of the territory. Understanding flight in *H. halys* is crucial to understanding the dispersal capacity and developing forecasting models for this pest. The purpose of this research was to assess the effects of starvation, age, mating status, sex, and preflight weight on flight parameters of laboratory-reared *H. halys* using computer-monitored flight mills. The mean flight distance observed over a 24-h period was 266 m and the maximum distance was 7.3 km. Overall, the flight capacity of males and females was similar, even though females weighed more than males. The proportion of *H. halys* that initiated flight was not affected by starvation, age, or mating status. The number of bouts of individual flights and velocity significantly increased with longer durations of starvation. The number of bouts significantly decreased with increasing age. The total distance flew and total flight time was not affected by starvation, age, or mating status suggest that *H. halys* are remarkably resilient, which may contribute to their success as an invasive species. The findings of this study could help better predict the dispersal potential of *H. halys* in Minnesota.

Key words: Halyomorpha halys, flight mill, invasive species, dispersal, stink bug

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly known as the brown marmorated stink bug, is an invasive species native to East Asia first introduced into the United States in the mid-1990s (Hoebeke and Carter 2003). This insect is polyphagous, with >300 reported host species, including many of agricultural importance (e.g., tree fruits, vegetables, and row crops) (Kuhar et al. 2012, Rice et al. 2014, Bergmann et al. 2016). Halyomorpha halys is a highly mobile pest, able to fly >70 km per day and easily move throughout the landscape in pursuit of preferred host plants (Lee et al. 2014, Wiman et al. 2014, Lee and Leskey 2015). Based on human-mediated transport and innate dispersal capacity, *H. halys* has rapidly spread throughout the globe, including much of North America (e.g., 46 U.S. states and four Canadian provinces), western and eastern Europe, and South America (e.g., Chile) (Haye et al. 2015, Faúndez and Rider 2017, Kriticos et al. 2017, Leskey and Nielsen 2018, Acebes-Doria et al. 2020, NIPMC 2020).

Since its introduction in the United States, feeding from *H. halys* caused the most significant documented agricultural losses in the mid-Atlantic region. A widespread outbreak within this region in 2010 resulted in a ~US\$37 million loss in apples and caused severe damage in many other specialty and row crops (Leskey et al. 2012, Leskey and Nielsen 2018). Additionally, feeding damage and economic losses are being reported in the Pacific Northwest and Midwestern United States (NIPMC 2020). The high dispersal capacity, polyphagy, and inter-species competition of *H. halys* is causing growing concern throughout the Midwestern United States, where its status ranges from recently detected or nuisance pest only (i.e., North Dakota, Nebraska, Kansas, Iowa, and Missouri) to an agricultural and nuisance pest of moderate (i.e., Ohio, Indiana, Illinois,

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Wisconsin, and Minnesota) to severe (i.e., Michigan) status (NIPMC 2020).

In Minnesota, *H. halys* was first detected in 2010 (Koch 2014). Within the last decade, it has been primarily a nuisance invader of homes and buildings (MDA 2020). Since 2015, mature and immature stages of *H. halys* detected in apple orchards and soybean fields have indicated the presence of breeding populations (Pezzini et al. 2019, Koch and Aita 2019). Given the diversity of agronomic and horticultural crops at risk, *H. halys* is an emerging threat to Minnesota; if populations continue to increase and spread as the insect has in other states, significant agricultural losses are likely to occur in the future. Therefore, it is pivotal to understand the dispersal capacity of *H. halys* to mitigate future economic losses.

Dispersal is a critical component for understanding the dynamics and ecology of pest populations (Stinner et al. 1983, Naranjo 2019). Understanding dispersal behavior is crucial for the development of monitoring and management tactics (Zhang et al. 2009, Lee et al. 2014, Lee and Leskey 2015, Grettenberger and Joseph 2019) as well as developing dispersal and forecasting models (Hudgins et al. 2017, Lustig et al. 2017). Various techniques have been used to study the dispersal and movement of H. halys in field settings, such as harmonic radar (Lee et al. 2014, Morrison et al. 2016, Kirkpatrick et al. 2019), mark-release-recapture using fluorescent dusts (Rice et al. 2015); protein markers (Blaauw et al. 2016), and the use of blacklight traps (Nielsen et al. 2013). Tracking insect movement in the field would be the ideal method for examining dispersal behavior; however, dispersal remains one of the most challenging and intractable processes to accurately characterize in the field (Naranjo 2019). Low recapture rates and limited distance and duration of tracking devices make it impractical to assess long-distance dispersal of insects using some of the methods described above (Yamamura et al. 2003, Franzén and Nilsson 2007, Lopez et al. 2017).

Laboratory tethered flight mills are relatively simple and inexpensive tools for measuring flight characteristics that are difficult to capture in the insect's natural habitat (Minter et al. 2018, Naranjo 2019). Although flight mills have limitations and do not truly represent natural flight conditions (Minter et al. 2018, Naranjo 2019), they are effective tools in determining how multiple experimental treatments can potentially affect the relative flight capacity of individuals (Taylor et al. 2010, Lopez et al. 2014). Flight mills have been used to determine how variable traits such as age (Sarvary et al. 2008, Zhang et al. 2008), feeding status (Fahrner et al. 2014, Wong et al. 2018), sex (Hughes and Dorn 2002, Kees et al. 2017), weight (Lopez et al. 2014, Lee and Leskey 2015), and reproductive status (Colvin and Gatehouse 1993, Schumacher et al. 1997) affect the flight capacities of insects.

To date, there have been a limited number of studies that have examined the flight capacities of pentatomid pests using flight mills. A recent study by Babu et al. (2020) assessed the baseline flight potential of the brown stink bug, Euschistus servus (Say) (Hemiptera: Pentatomidae), across multiple hosts and found that the longest distance flown by an individual was approximately 16 km and the higher mean dispersal occurred soon after overwintering emergence. In previous work with H. halys, the baseline flight potential of tethered field-collected adults showed that approximately 85% of individuals flew up to 5 km in 24 h, and the longest distance flown by an individual was 117 km (Wiman et al. 2014, Lee and Leskey 2015). To our knowledge, few studies have examined how variables manipulated under controlled laboratory conditions affect the flight capacity of H. halys on flight mills. Therefore, the objectives of this study were to assess the effects of starvation, age, mating status, sex, and preflight weight on flight parameters of laboratory-reared *H. halys*, including distance flown, total flight time, flight velocity, and bouts of flight (i.e., number of flight events of an individual). Findings from this study could help better predict the dispersal capacity of *H. halys*, enhancing risk assessment analyses for future range expansion of this pest.

Materials and Methods

Insects

Halyomorpha halys adults were reared in a laboratory colony at the University of Minnesota. The colony was first established in 2012 with individuals collected from a residence in Wyoming, MN (Govindan and Hutchison 2020). In the fall of each year, additional individuals were collected from this location, subjected to diapause conditions in the laboratory and added to the colony to preserve genetic diversity (Govindan and Hutchison 2020). Mixed-sex groups of 40-50 H. halvs were maintained in $34 \times 34 \times 61$ -cm mesh rearing cages (BioQuip Products, Rancho Domingues, CA, USA) at ~25°C, ~70% RH, and a 16:8 (L:D) photoperiod in walk-in laboratory rearing rooms. Insects were provisioned with an ad libitum supply of dried raw organic sunflower (Helianthus annuus L.) (Asterales: Asteraceae) and soybean (Glycine max (L.) Merr.) (Fabales: Fabaceae) seeds, carrots (Daucus carota subsp. sativus (Hoffm.) Schübl. & G. Martens) (Apeales: Apeaceae), and pods of green beans (Phaseolus vulgaris L.) (Fabales: Fabaceae) (Dingha and Jackai 2017). To maintain humidity, cages were misted with deionized water every weekday. In addition, each cage was provided with flats of vegetative-stage green bean plants (V2-V3 stage) to serve as an ovipositional substrate (Shanovich et al. 2020). Egg masses were collected on a daily basis from the underside of the green bean leaves and placed in 55 × 15-mm Petri dishes (Fisher Scientific, Pittsburgh, PA, USA) with a half piece of moistened 5.5-cm-diameter filter paper (Fisher Scientific, Pittsburgh, PA, USA). Petri dishes were held in a growth chamber (Percival Scientific, Inc., Perry, IA, USA) under similar environmental conditions as the walk-in laboratory rearing rooms, and eggs were reared to second instar nymphs. The second instars were then transferred to mesh cages (31×31×31-cm) and reared under the same conditions as described for the adults. Upon eclosion, newly emerged adults (i.e., ≤24-h old) were maintained according to the specifications of each experimental protocol described below.

Flight Mills

Twenty-two computer-monitored flight mills were used to investigate the flight capacity of H. halys. Detailed information for flight mill construction (i.e., design, data acquisition, and output) can be found in Fahrner et al. (2014) and Kees et al. (2017). The flight mills were spaced ~30 cm from each other and the surfaces surrounding the equipment were white to minimize potential orientation cues for the insects. Insects were attached to the tip (bent 90°) of an 8-cm tether arm (solid 33-gage [American] copper wire) using a droplet of glue (Surebonder, Wauconda, IL) from a low-temperature glue gun (Stanley, Jackson, TN), gently pressed against the center of H. halys pronotum. This attachment method was similar to that used for field-collected H. halys by Wiman et al. (2014) and Lee and Leskey (2015). After attachment, but before placement on the flight mills, the hemelytra of H. halys were carefully examined to ensure that wing movement was not noticeably inhibited by the tether arm or glue. To ensure a proper balance of the tether arm on the flight mill, a counterbalance was added to the opposite end. Once the insects were successfully attached, the tether arm was placed into the flight mill to begin recording data.

All movements of the tether arm, including actual flight activity and pseudo-movements due to air current or accidental bumps during trial initiation, were recorded by an infrared (IR) sensor. The pseudo-movements can be identified and omitted to avoid misleading results (Fahrner et al. 2014, Kees et al. 2017). To exclude potential spurious recordings, thresholds for flight speed and bouts of flight were implemented. Following the initial flight, a limit for maximum speed was set at 2 m/s (7.2 km/h) and a minimum of twelve revolutions were set as criteria for counting flights. These thresholds were set following personal observations of *H. halys* during a tethered flight.

Experiments

All flight experiments took place at room temperature of ~24°C, ~55% RH, and were of 24-h duration in constant light (2,700K fluorescent; ~1955 lux). We recognize potential concerns about performing such experiments under artificial light sources (Babu et al. 2020); however, the methodology including lightning, was the same as that used successfully by Fahrner et al. (2014) and Kees et al. (2017). Individual insects were not re-used for other experiments after a 24-h flight trial. For all experiments, insects were weighed to the nearest 0.001 g using an analytical balance (Sartorius Entris 224, Sartorius AG, Göttingen, Germany). In addition, the sex of H. halys was recorded for all experiments after the methods of Vétek et al. (2014). A maximum of 22 insects could be flown on a given day, so each experiment was carried out over multiple days (i.e., temporal blocks). Three separate experiments were conducted to test the effects of starvation (i.e., number of days without food), age (i.e., number of days after emergence of adults), and mating status (i.e., unmated vs. mated) on the flight capacity of H. halys.

Experiment 1-Effects of Starvation on H. halys

To determine the effects of starvation on the flight capacity of H. halys, groups of mixed-sex adults were selected from the laboratory colony. Insects were of uniform age, collected approximately 7d after the emergence of adults. These adults were transferred into circular 18.5 × 8-cm plastic dishes (Pioneer Plastics, Inc., North Dixon, KY) with a circular 9.0-cm-diameter filter paper (Fisher Scientific, Pittsburgh, PA) and provided water ad libitum through moistened cotton wicks. Each dish with insects was randomly assigned to one of four durations of starvation: 0 (i.e., no starvation), 1, 3, and 7 d. Dishes were held in a growth chamber (Percival Scientific, Inc., Perry, IA) under the same environmental conditions as the laboratory colony for the desired duration of starvation. To have individuals from each starvation duration ready to fly on the same day, food was provided to the individuals from the time of set up of the dishes for the entire 7 d (i.e., 0-d starvation), for the first 6 d (i.e., 1-day starvation), for the first 4 d (i.e., 3-day starvation), or no food provided over the entire 7 d (i.e., 7-day starvation). In total, 319 H. halys were placed on the flight mills for this experiment.

Experiment 2-Effects of Age on H. halys

To determine the effects of age postemergence on flight capacity of *H. halys*, newly emerged second instars were transferred to individual mesh cages and held in a growth chamber under the same environmental conditions and food provisioning as the laboratory colony described earlier. Each cage contained a cohort of mixed-sex *H. halys* adults of the same age. Age groups ranged between 3 and 47 d postemergence of adults and insects from multiple age groups were randomly assigned across the 22 positions on the flight mill on a given day. In total, 106 *H. halys* were placed on the flight mill.

Experiment 3-Effects of Mating Status on H. halys

To determine the effects of mating status on flight capacity of H. halys, individuals were assigned upon emergence as adults to two groups: unmated (i.e., males and females were kept isolated from one another for 15 d in preparation for the experiment) or mated (i.e., male-female pairs were kept together for 15 d in preparation for the experiment and visually checked daily to ensure that mating occurred). Both groups were placed inside 100 × 20-mm Petri dishes (Fisher Scientific, Pittsburgh, PA) with ~25 0.5-mm diameter holes in the lid for ventilation. A 9.0-cm-diameter piece of filter paper (Fisher Scientific, Pittsburgh, PA, USA) was placed in the dish and moistened as needed, and food was provided by adding fresh organic green beans, carrots, and dry soybean seeds every two to three days. Females and males were flown on separate days. Immediately following a 24-h flight trial and weighing of individuals, adults were placed into the freezer at approximately -20°C for later dissection. Females were dissected in Dulbecco's Phosphate Buffered Saline 1X (DPBS) with calcium and magnesium solution (Meditech Inc., Manassas, VA) to record the mating status. To determine mating status, spermatheca condition was examined following methodologies from Cullen and Zalom (2006) and Nielsen et al. (2017), which described virgin females having a slender and translucent spermatheca and mated females having an enlarged and opaque spermatheca. In total, 141 H. halys were placed on the flight mill, with between 28 and 40 individuals for each mating status of each sex.

Data Extraction and Statistical Analyses

The recording of the flight parameters such as the number of rotations per flight and flight duration was performed using customized LabVIEW software (National Instruments Corporation, Austin, TX). The extraction of flight metrics from the raw phase-change data followed methodologies described by Fahrner et al. (2014) and Kees et al. (2017). Flight variables calculated to characterize the flight capacity of *H. halys* were total flight distance, time, velocity, and bouts. The use of more than one response variable is appropriate for flight mill experiments, because using a single variable might fail to reveal flight differences or can lead to misleading results (Dingle 1985, Luo et al. 2002).

All data were analyzed using R version 4.0.0 (R Core Team 2020). Linear mixed-effect models incorporating terms for electronic flight channel and day of the trial as random effects were used for most analyses. Three separate analyses on the preflight weight of the insects were conducted. First, ANCOVA was used to analyze the effect of starvation (covariate), sex (factor), and their interaction on preflight weight. Second, ANCOVA was used to analyze the effect of age (covariate), sex (factor), and their interaction on preflight weight. The previous two analyses were performed using the function "Imer" from package "Ime4" (Bates et al. 2015). Finally, a two-way ANOVA was used to analyze the effect of sex (factor), mating status (mated vs unmated, a factor), and their interaction on preflight weight. This analysis (ANOVA type II) was performed with χ^2 tests to test the significance of terms using the function "Anova" from package "car." Means were compared by obtaining least-square means using the function "Ismeans" from package "Ismeans" (Lenth 2016) adjusted for Tukey's HSD test. A term for preflight weight showed high correlation coefficients (≥ 0.7) with other predictor variables (i.e., the effect of starvation, age, mating status, and sex) described in models below, therefore the term for preflight weight was not included as an additional predictor in those analyses.

ANCOVA was used to analyze the effect of starvation (covariate), sex (factor), and their interaction on several separate response variables of interest (i.e., 1) distance flown; 2) total flight time; 3) bouts of flight; 4) flight velocity). For each response variable, separate mixed-effect model ANCOVAs were performed, with random effects as previous. The effect of age on the flight-related response variables was analyzed the same as for the effect of starvation, except with age as the covariate. A two-way ANOVA was used to analyze the effects of mating status (factor), sex (factor), and their interaction on the flight-related response variables.

Three separate analyses on the probability of insects flying were conducted using generalized linear mixed-effect models with the function 'glmer' from the package "lme4," with a binomial distribution for the response variable, logit as the link-function, and random effects as specified above. First, ANCOVA was used to analyze the effect of starvation (covariate), sex (factor), and their interaction on the probability of insects flying. Second, ANCOVA was used to analyze the effect of age (covariate), sex (factor), and their interaction on the probability of insects flying. Finally, a two-way ANOVA was used to analyze the effect of sex (factor), mating status (factor), and their interaction on the probability of insects flying.

For all linear mixed-effects models, graphical examinations of residual plots were used to check assumptions of normality and homoscedasticity of the errors. To meet model assumptions, some response variables (i.e., distance flown, total flight time, flight velocity, and bouts of flight) were subjected to variance-stabilizing logarithmic transformations. For all analyses, nonsignificant interaction terms (P > 0.05) and subsequent insignificant main effects were removed for model simplicity and to the simplify graphical presentation. Logarithmic model predictions and 95% CIs were back-transformed using the exponential function and are reported in the text.

Results

General Flight Performance

The flight parameters of *H. halys* across all three experiments are summarized in Table 1. Overall, 566 adults were flown during the three experiments (283 females, 283 males). From this total, 80% (n = 453) of the insects initiated flight; only 1% (n = 6) of the individuals had died at the end of a 24-h flight trial and were not included in the analysis. Among the individuals that initiated flight, the frequency distribution for total flight distance for both sexes was highly right-skewed with 93.1% (n = 422) of individuals flying <1 km in 24 h. Approximately 7% (n = 31) of individuals flew >1 km. The mean flight distance was 266.51 ± 31.44 SEM m with a maximum distance flown of 7.3 km during a 24-h flight mill trial (Table 1). Total flight time ranged from 11.4 s to 3.6 h (6.57 ± 0.85 SEM min), and mean flight velocity was 0.64 ± 0.01 SEM m/s (Table 1). A number of flight bouts ranged from 1 to 25 (3.57 ± 0.16 SEM) (Table 1).

 Table 1. Summary of flight parameters calculated for all three experiments of *H. halys* tested on flight mills. Only insects that flew were included in the analysis. Insects were placed on the flight mill for 24 h and were not re-used

<i>n</i> = 453	Distance (m)	Total flight time (min)	Velocity (m/s)	Bouts
Minimum	6	0.19	0.23	1.0
Median	48	1.30	0.55	2.0
Mean	266	6.57	0.64	3.6
Maximum	7269	216.11	1.45	25.0

Effects of Starvation on H. halys

Of the 319 *H. halys* placed on flight mills for this experiment, 245 individuals (76.8%) initiated flight (128 females, 117 males). For the starvation experiment, preflight weights of females were significantly heavier (0.193 ± 0.005 g) than males (0.119 ± 0.002 g) (*F* = 103.07; df = 1, 62; *P* < 0.001). Prior to flight, weight decreased with increasing duration of starvation (*F* = 100.28; df = 1, 122; *P* < 0.001), and the rate of weight loss varied between sexes (*F* = 30.28; df = 1, 118; *P* < 0.001) (Fig. 1a). Females lost weight at a rate of 10 ± 1 mg/d, while males only lost 3 ± 1mg of weight per day when food was withheld (Fig. 1a).

The proportion of *H. halys* that initiated flight was not affected by duration of starvation, sex of individuals or the interaction of duration of starvation and sex (P > 0.05). The number of bouts of flight during a 24-h period was significantly affected by duration of starvation (F = 5.60; df = 1, 191; P = 0.018), with number of bouts increasing with longer durations of starvation (1.04 bouts/d; 95% CI [1.00, 1.09]) (Fig. 2c). However, number of bouts was not affected by sex or the interaction between duration of starvation and sex (P >0.05). Although flight velocity was affected by duration of starvation (F = 6.16; df = 1, 220; P = 0.013) and sex (F = 14.54; df = 1, 98;P < 0.001), the interaction was not significant (P > 0.05) (Fig. 2d). Across durations of starvation, males flew 0.088 m/s faster (95% CI [0.083, 0.093]) than females and for both sexes a 1-d increase in duration of starvation was associated with 1.02 m/s increase (95% CI [1.00, 1.03]) in flight velocity (Fig. 2d). Total distance flown (Fig. 2a) and total flight time (Fig. 2b) were not affected by duration of starvation, sex, or the interaction (P > 0.05).

Effects of Age on H. halys

Of the 106 H. halys placed on flight mills for this experiment, 94 individuals (88.7%) initiated flight (44 females, 50 males). For the age experiment, preflight weights of females were significantly heavier $(0.238 \pm 0.005 \text{ g})$ than males $(0.130 \pm 0.001 \text{ g}; F = 361.2; \text{ df} = 1,$ 103; P < 0.001). Prior to flight, weight increased as individuals grew older (F = 12.00; df = 1, 78; P < 0.001). Females gained weight at a rate of 1.5 ± 0.3 mg/d and males gained weight at a rate of $0.2 \pm$ 0.1 mg/d (*F* = 6.30; df = 1, 98; *P* = 0.013) (Fig. 1b). The proportion of H. halys that initiated flight was not affected by age, sex of individuals or the interaction of age and sex (P > 0.05). Number of bouts was not affected by sex or the interaction of sex and age (P >0.05). However, number of bouts was significantly affected by age (F = 8.14; df = 1, 88; P = 0.0053), with the number of bouts decreasing by 0.98 bouts per day with age (95% CI [0.97, 0.99]) (Fig. 3c). Total distance flown (Fig. 3a), total flight time (Fig. 3b) and flight velocity (Fig. 3d) were not affected by the age of individuals, sex, or the interaction (P > 0.05).

Effects of Mating Status on H. halys

Of the 141 *H. halys* placed on flight mills for this experiment, 114 individuals (80.8%) initiated flight (63 females, 51 males). For the mating status experiment, preflight weights of females were significantly heavier (0.219 ± 0.003 g) than males (0.119 ± 0.001 g; F = 233.06; df = 1, 8; P < 0.001). Prior to flight, weight of individuals were significantly affected by mating status ($\chi^2 = 14.54$, df = 1, P < 0.001), and by the interaction of mating status and sex of individuals ($\chi^2 = 12.76$, df = 1, P < 0.001) (Fig. 1c). Mated females were heavier (0.230 ± 0.005 g) than unmated females (0.207 ± 0.004 g), while for males the preflight weight of mated (0.1189 ± 0.002 g) and unmated (0.1193 ± 0.002 g) were not statistically different (P > 0.05) (Fig. 1c). The proportion of *H. halys* that initiated flight was



Fig. 1. Effects of duration of starvation (a), age postemergence (b), and mating status (c) of adult *H. halys* on preflight weight of males and females. For (a) and (b), solid lines represent statistically significant trend lines and shaded regions represent a 95% confidence interval for model predictions. For (c), dark gray bars represent females and light gray bars represent males, and treatment means with the same letter are not significantly different (Tukey HSD, *P* > 0.05).



Fig. 2. Effect of duration of starvation of *H. halys* adults on (a) total distance flown, (b) total flight time, (c) number of bouts, and (d) velocity over 24-h flight trials. Solid lines represent statistically significant trend lines. Shaded regions represent a 95% confidence interval for model predictions. Only individuals that flew were included in these analyses (n = 245).



Fig. 3. Effect of age postemergence of *H. halys* adults on (a) total distance flown, (b) total flight time, (c) number of bouts, and (d) velocity over 24-h flight trials. Solid lines represent statistically significant trend lines. Shaded bands represent a 95% confidence interval for model predictions. Only individuals that flew were included in these analyses (*n* = 94).

not affected by mating status, sex, or the interaction of mating status and sex of individuals (P > 0.05). Mating status did not affect the response variables in this study (i.e., total distance flown, number of bouts, total flight time, and flight velocity) (P > 0.05).

Discussion

Halyomorpha halys is a significant nuisance and agricultural pest that is highly mobile and can rapidly spread throughout the landscape. Although some statistical differences were seen across our experiments, especially for number of flight bouts and velocity, these differences likely represent minimal ecological significance. These results show that *H. halys* are remarkably resilient and demonstrate little to no increase or decrease in flight capacity with food deprivation, advancing age, or mating status, which likely contributes to their emergence as a highly successful invasive species.

In contrast to our results, some studies with other insects have shown increasing movement over an initial period of starvation followed by a decrease in movement due to mortality, lack of energy reserves, or other chronic stresses as starvation continues (Elsey 1974, Fadamiro and Wyatt 1995, Perez-Mendoza et al. 2011, Scharf 2016). One study investigated the aspects of flight metabolism on two stink bug species, *Encosternum delegorguei* Spinola (Hemiptera: Tessaratomidae) and *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae), and found that during intensive flight activity lipid oxidation in the flight muscles is the main source of energy production (Gäde and Marco 2009). If durations of food deprivation greater than one week were used in the present study, *H. halys* would likely have shown an eventual decrease in flight capacity. The increasing number of bouts with increasing duration of starvation indicates the insects were stopping and restarting flight more frequently as their hunger increased, which likely increases their success in finding food across a landscape.

Individuals used in the present experiment ranged from 3 to 47 d old, but laboratory-reared *H. halys* males and females can survive for approximately 60 and 85 d at 27°C, respectively (Govindan and Hutchison 2020). If individuals older than those flown in this study had been used, the results may have shown an eventual decrease in flight capacity, as has been shown with other insects. For example, Maharjan and Jung (2009) compared flight parameters of the bean bug, *Riptortus clavatus* (Thunberg) (Hemiptera: Alydidae) in relation to its age and found that flight activities were higher in 25–30 d old individuals compared to 5 and 45 d old individuals. Similarly, for three species of *Adelphocoris* (Hemiptera: Miridae), which are pests of cotton in China, the flight capacity of individuals was low for 1-d old adults, gradually increased with age until 10- to 13-d old,

and then gradually decreased until 25-d old individuals (Lu et al. 2009). The number of bouts decreased with increasing age, which may suggest that older *H. halys* are more prone to preserve energy resources that would otherwise be used for more frequent flights. However, Wiman et al. (2014) observed that overwintered individuals performed a higher number of flights (bouts) than individuals from the summer generation, suggesting that factors other than age alone (e.g., host and mate searching, and energy reserves) may influence the number of bouts for individuals in the wild.

Impacts of mating status on flight capacity seem to be species-specific. In this study, the mating status of females and males had no significant effect on the flight capacity of *H. halys*. Similarly, mating status of males and females had little to no effect on the flight performance of three *Adelphocoris* spp. (Heteroptera: Miridae) (Lu et al. 2009). In addition, Lu et al. (2007) found no significant effect of mating status on flight capacity of *Lygus lucorum* (Meyer-Dür) (Heteroptera: Miridae).

Flight performance of insects generally tends to increase as the percentage of body weight comprised of flight muscle increases (Lopez et al. 2017). However, the size of flight muscles may fluctuate based on varying biotic and abiotic factors and therefore might not always be correlated with body size (Marden 2000, Lopez et al. 2017). The experimental manipulations (e.g., starvation, age, and mating status) performed in this study affected H. halys body mass and therefore were expected to impact the flight capacity of this insect. In addition, female Pentatomidae are generally larger than their male counterparts (Panizzi et al. 2000), which corroborates the higher preflight body weights found for female H. halys in the present experiments (Fig. 1). However, the relatively larger females in our experiments did not demonstrate higher flight capacity than the males, perhaps due to the fact that body weight also includes lipid content, water content, and nonflight musculature (Shelton et al. 2006, Lopez et al. 2014). Lu et al. (2019) actually found that males of the sycamore lace bug, Corythucha ciliata (Say) (Hemiptera: Tingidae) had significantly greater flight speed than females over a 24-h flight mill study even though the females were heavier than males.

While laboratory flight mill studies provide useful insights regarding several variables that are difficult to quantify in the field, appropriate caution must be exercised when interpreting flight mill data (Riley et al. 1997, Taylor et al. 2010, Naranjo 2019). Limiting factors such as handling and tethering of insects, lack of natural stimuli, increased weight load, and reduced energy expenditure of tethered insects can impact results; therefore, it is important to design experiments that use a comparative approach (Wong et al. 2018). We investigated the flight capacity of H. halys through a comparative process focusing on the relative changes in behavior due to specific biotic factors that we manipulated while controlling other aspects of rearing and the flight mill process, as suggested by Naranjo (2019) for laboratory-reared individuals. Distances flown for the laboratory-reared H. halys in this experiment were generally lower than those observed for wild-collected H. halys (Wiman et al. 2014, Lee and Leskey 2015) and other wild-collected Pentatomidae (Babu et al. 2020) assessed on flight mills. Such differences between laboratory-reared and wild-caught individuals have been observed for other insects (Baker et al. 1980, Nakamori and Simizu 1983, McKibben et al. 1988). Despite this, we chose to use laboratory-reared individuals, so we could carefully control the rearing conditions to allow manipulation and comparison of the effect of certain factors.

Individuals used in this study, which originated from individuals collected in Minnesota (see Materials and Methods section) may represent a distinct population from those used in previous *H. halys* flight studies. For example, this Minnesota colony developed faster and survived at higher rates compared to eastern populations (Govindan and Hutchison 2020). Understanding the biological attributes of local *H. halys* dispersal is key to the improvement of integrated pest management strategies to successfully manage this pest in Minnesota, especially exploiting strategies that depend on insect movement such as the attract-and-kill strategy (El-Sayed et al. 2009, Morrison et al. 2019). Future research will use data from this study to parametrize dispersal models for *H. halys* to help predict its spread throughout the state.

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