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Author(s): Samuel J. Fahrner, Jonathan P. Lelito, Karen Blaedow, George E. Heimpel, and Brian H. Aukema

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Factors Affecting the Flight Capacity of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a Classical Biological Control Agent of *Agrilus planipennis* (Coleoptera: Buprestidae)

SAMUEL J. FAHRNER,^{1,2} JONATHAN P. LELITO,³ KAREN BLAEDOW,⁴ GEORGE E. HEIMPEL,¹
AND BRIAN H. AUKEMA¹

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ABSTRACT The dispersal characteristics of a biological control agent can have direct implications on the ability of that agent to control populations of a target host. *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) is a parasitic wasp native to eastern Asia that has been introduced into the United States as part of a classical biological control program against the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). We used computer-monitored flight mills to investigate the role of age, feeding status, mating status, and size on the flight capacity of female *T. planipennisi* over a 24-h period. We also compared flight capacity between sexes. Flight distance of female *T. planipennisi* representative of populations released in the biological control program averaged 1.26 km in 24 h with a maximum flight of just over 7 km. Median flight distance, however, was 422 m. The flight capacity of females fed a honey–water solution was 41× that of females provided only water, who flew very little. Larger females were capable of flying farther distances, but age did not affect the flight capacity of females up to 70 d posteclosion. Females dispersed 6× farther than did their smaller, male counterparts. The implications of our findings to host–parasitoid interactions and release protocols for distributing *T. planipennisi* are discussed.

KEY WORDS dispersal, koinobiont, biological control, wood-borer, *Fraxinus* spp.

The ability of a biological control agent to establish in a novel environment and control populations of an invasive pest depends, in part, on the dispersal characteristics of that agent (Hopper and Roush 1993, Heimpel and Asplen 2011). Knowledge of the flight capability of a biological control agent may aid in optimizing release strategies in both classical and augmentative biological control programs, such as fine-tuning spatial and temporal distances between release points, optimizing release densities, and minimizing negative impacts from Allee effects at low densities (Hopper and Roush 1993, Shea and Possingham 2000). Optimization may be particularly important when there are limitations on the number of insects available for release. Variability in flight potential may interact with factors such as host density, environmental stochasticity, and the availability of biological control agents to influence coverage, establishment, and efficacy. Dispersal remains one of the most challenging variables to characterize, however, particularly for insects of small

size in natural settings. In the early stages of a classical biological control program, released insects may exist at low densities and, therefore, can be difficult to sample effectively.

The emerald ash borer *Agrilus planipennisi* Fairmaire (Coleoptera: Buprestidae) is an invasive wood-boring beetle native to eastern Asia that was discovered near Detroit, MI, in 2002 as the cause of widespread mortality of ash trees (*Fraxinus* spp.; Haack et al. 2002, Cappaert et al. 2005, Poland and McCullough 2006, Herms and McCullough 2014). The invasive range of emerald ash borer includes non-contiguous populations from the eastern to southeastern United States west to Colorado, including populations along the southern border of central Canada. The range continues to expand due to natural dispersal by the beetle and anthropogenic movement of infested host material (Haack et al. 2002, Muirhead et al. 2006, Poland and McCullough 2006, Mercader et al. 2009). The larvae of emerald ash borer damage and kill host trees by excavating galleries in the phloem and outer xylem, which stymies the translocation of nutrients (Poland and McCullough 2006). In China, emerald ash borer is typically associated with stressed ash trees. In eastern North America, almost all *Fraxinus* spp. appear highly susceptible to insect colonization (Cappaert et al. 2005, Poland and McCullough 2006, Rebek et

¹ Department of Entomology, University of Minnesota, 219 Hodson Hall, 1980 Folwell Ave., St. Paul, MN 55108.

² Corresponding author, e-mail: fahr0051@umn.edu.

³ USDA APHIS PPQ, Emerald Ash Borer - Biological Control, 5936 Ford Court Suite 200, Brighton, MI 48116.

⁴ USDA-ARS Tree Fruit Laboratory, 1104 N. Western Ave., Wenatchee, WA 98801.

al. 2008). Moreover, native predators and parasitoids are not causing sufficient mortality to reduce populations of emerald ash borer to nondamaging levels. Given the rapid range expansion of emerald ash borer over the past decade, this invasion poses a serious threat to maintaining ash as a forest and urban landscape tree (Cappaert et al. 2005, Gandhi and Herms 2010).

In response to this invasion, a classical biological control program began in 2003 with a survey for natural enemies in China (Liu et al. 2003). This program has resulted in the introduction of three Hymenopteran parasitoids from China into the United States including the gregarious larval endoparasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Bauer et al. 2008, Herms and McCullough 2014). Adult *T. planipennisi*, which measure 4 mm in length, likely locate endophytic host larvae using ash leaf volatiles to identify potentially infested trees. Feeding vibrations and chemical cues emitted by host larvae are likely exploited to locate suitable hosts under the bark, although the exact mechanisms are still being elucidated (Ulyshen et al. 2011). *T. planipennisi* prefer to oviposit in fourth-instar host larvae but can attack other instars (Liu et al. 2007, Ulyshen et al. 2010).

Upon locating a suitable host, female *T. planipennisi* insert their ovipositor through the bark, penetrate the integument of the host larva, and oviposit into the host (Liu et al. 2007, Ulyshen et al. 2010, Duan et al. 2011). Mean lifetime fecundity for *T. planipennisi* is 57 progeny per mated female (range: 15–108) with a 3:1 (female: male) sex ratio (Duan et al. 2011). Parasitoid larvae develop for 7–10 d during which time they typically consume the entire host except for the integument (Duan et al. 2011). At that time, the larvae begin a wandering stage, eventually pupating before the emerging wasp chews an exit hole through the bark (Yang et al. 2006, Duan et al. 2011). Development from egg to adult life stages takes ≈ 27 d (Duan et al. 2011). *T. planipennisi* has approximately four generations per year in China and is expected to have multiple generations per year in areas of establishment throughout the American Midwest (Yang et al. 2006, Liu et al. 2007, Duan et al. 2011). *T. planipennisi* has established at several locations in Michigan, and spread rates are estimated at 1–5 km per year (Duan et al. 2013).

Several techniques have been used to study the movement of insects, such as mark–release–recapture with fluorescent dyes, etchings, genetic markers or other noninvasive labels (Hagler and Jackson 2001), harmonic radar (Mascanzoni and Wallin 1986, Machial et al. 2012), airplanes (Jackson et al. 2008), flight chambers (Stinner et al. 1983), and tethered flight apparatuses (Stinner et al. 1983). Each method has associated advantages and disadvantages, the discussion of which is beyond the scope of this article. Given size, endophytic life history, and limitations on the number of female *T. planipennisi* available for mark–release–recapture studies, we use computer-monitored flight mills to

study the flight capacity of *T. planipennisi*. Flight mills are effective tools for determining how experimental treatments such as age (Rowley and Graham 1968, Schumacher et al. 1997), mating status (Armes and Cooter 1991, Lu et al. 2007), feeding status (Clements 1955, Wanner et al. 2006), size (Wu et al. 2006, Sarvary et al. 2008), temperature and humidity (Zhang et al. 2008), sex (Stewart and Gaylor 1994), and parasitism (Zhang et al. 2009a, 2012) affect relative flight capacity. Here, we investigate how several variables affect the flight capacity and postflight survival of *T. planipennisi* in the laboratory. For female wasps, these variables include age, mating status, feeding status, and size. Males are only flown for comparisons with female flight. Our goal is to provide knowledge that may aid in optimizing release strategies and establishment for *T. planipennisi*.

Materials and Methods

Flight Mill. Twenty-four computer-monitored flight mills were used to investigate the flight capacity of *T. planipennisi*. The design of the flight mill was similar to other mills used to fly minute insects, with 1) an insect attached to a counterbalanced tether arm, and 2) an electronic sensor that detects when the insect flies in a circle about a low-friction vertical axis connected to the middle of the tether arm (Taylor et al. 1992, Zhang et al. 2008, Evenden et al. 2014). In this experiment, the ends of fixed flight mill arms were attached perpendicularly to the top and bottom of a support rod 20 cm in length, forming a block “C”-shape. At the open end of each fixed flight mill arm, an 8-cm cylinder housing a circular Rare-Earth magnet (diameter: 0.635 cm, height: 0.254 cm, pull force: 1.13 kg) was attached such that there was a 4-cm gap between the two cylinders. Here, the mobile part of the flight mill, the tether arm, was placed and able to rotate. The magnets were arranged with opposite polarities facing each other, creating a magnetic field to hold a stainless steel No. 1 insect pin. This pin served as the axial needle for the tether arm. The tether arm was constructed by attaching the middle of an 11-cm piece of 108 American wire gauge (diameter: ≈ 0.171 mm) copper wire perpendicularly to the middle of the insect pin, forming a cross. This resulted in two, even-length tether arms of 5.5 cm each. The sharp end of the insect pin resting on the bottom magnet and held vertically by the magnetic field was the only source of friction. The insect pin was driven through the center of a circular encoder wheel with four equally spaced radial slits and weighing a mean of 0.345 g (± 0.0006 SE, $N = 27$). When the wheel rotated due to insect flight, an infrared (IR) slot sensor would detect phase changes of the encoder wheel, i.e., rates at which an IR beam in the sensor was broken by the encoder wheel. Data output was written to a 500 GB hard drive in real time on a dedicated 3.0 GHz computer. Extracting flight metrics from the raw phase change data were conducted

using R (R Core Team 2014) by dividing the number of phase changes by four (i.e., number of radial slits) and multiplying it by the circumference of the flight path. A flight was deemed complete when time between phase changes was >1 s for four consecutive seconds.

***T. planipennisi*: Rearing and Attachment to the Tether Arm.** All *T. planipennisi* were obtained from the Emerald Ash Borer Biological Control Production Facility in Brighton, MI (United States Department of Agriculture–Animal and Plant Health Inspection Service), and received as larvae or pupae within, or recently eclosed from, host larvae. To rear parasitoids to adulthood, one or two infested host larvae were placed in 32 oz. glass jars and held at 27°C and 75% relative humidity (RH) on a photoperiod of 16:8 (L:D) h. Unless experimental protocol required otherwise, such as in starvation or mating trials, parasitoids were fed a dilute honey–water solution through a mesh cloth fastened to the top of the rearing container, and males and females were housed in the same containers. One large drop of honey was provided daily to the mesh cloth on top of each container and the mesh cloth was then saturated with deionized water. When preparing a flight mill experiment, insects were retrieved at random from various containers using an aspirator and transferred individually to 0.5-ml microcentrifuge tubes. Care was taken to select parasitoids at various heights within the container to control for the potential of insects near the top of the container to be more apt to engage in flight. Insects were standardized by eye for size in experiments where size was not a treatment or was a potentially confounding variable such as in comparisons between the females and males, as females are significantly larger than males (Yang et al. 2006).

For attachment to the tether arm, insects were gently coerced from the microcentrifuge tube onto an icepack. The ice pack was removed from the freezer for several minutes to prevent cold shock to the insects. Once an insect was sufficiently chilled, the tip of the copper tether arm was dipped in a droplet of cyanoacrylate super glue (Loctite Super Glue Gel; Henkel Corporation) and lightly pressed against the dorsal surface of the parasitoid's thorax such that the wire and surface of the insect were perpendicular. This glue was effective at securing the insects, and previous studies in our own laboratory indicated no toxic effects for other insects over the course of up to 1 yr (Machial et al. 2012). The terminal 5 mm of the copper wire was then gently bent 90° so that the insect was facing perpendicular to the tether arm, resulting in a radius of 5 cm for the final tether arm.

Defining a Flight. Once the insect was attached to the tether arm, the tether arm was placed into the magnetic field. An insect was only included in the analysis if regular flight occurred at the onset of the trial, i.e., if the insect initiated flight within 30 s and that flight resulted in a minimum of three revolutions. Most insects initiated flight almost immediately after being placed on the flight mill. Following the initial flight, a minimum threshold of three full revolutions was used as a criterion for counting

flights to prevent small movements by the insect resulting in spurious flight recordings. We restricted flights as occurring at speeds between 0.5–1.8 km/h for treatments not specifically investigating size or potentially confounded by size, such as comparisons between male (1.6–2.2 mm) and female (2.7–4.1 mm) insects of different lengths (Yang et al. 2006). These thresholds were set following preliminary observations in the laboratory of this insect in tethered flight. We imposed upper and lower bounds on flight speed to preclude rare, spurious recordings where the insect happened to stop and align the border of the slot on the encoder wheel with the IR beam, potentially resulting in millisecond breaks and unrealistic flight speeds. The minimum velocity threshold was lowered to 0.36 km/h in male and female comparisons and experiments studying the effect of size on flight capacity, as smaller insects were occasionally slower. This lower threshold was also set using preliminary observations of *T. planipennisi* in tethered flight.

***T. planipennisi*: Experimental Treatments.** The effects of the following variables on the flight capacity of *T. planipennisi* were investigated: age (number of days posteclosion), mating status, feeding status (honey–water and water), size, and sex (male and female). Parasitoids were typically obtained from groups of four to six host larvae and were randomly assigned to treatments. Unless the experimental protocol required a specific change in parasitoid rearing protocol, all insects flown were female, younger than 35 d old, and provided a honey–water dilution for feeding ad libitum. That is, male parasitoids were flown for comparisons between sexes, insects older than 35 d old were flown for the age study, and a subset of wasps were only fed water (starved) upon eclosion for the fed and starved comparisons. All flight trials took place at room temperature (22.7°C \pm 0.03 SE) and humidity (20.2% RH \pm 0.43), and were of 24-h duration. A minimum of 45 insects were flown for each experiment (see Results for details). The survival status was determined once at the conclusion of each flight trial using movement of the wings or legs as an indicator of survival. Insects were not reused following a 24-h flight trial and stored at -20°C in 0.5-ml microcentrifuge tubes immediately following completion of the trial.

In studies of the effect of age on flight capacity, insects up to 75 d old were flown. Insects of varying ages were randomly assigned to channels of the flight mill on a given day. In studies on the effect of mating status on flight capacity, a subset of female pupae were separated from the newly arrived host larvae and held in separate jars. For comparisons between fed and unfed parasitoids, a hot anthrone test was used to determine the total sugar content of individual parasitoids postflight. Size was measured following a 24-h flight trial. For each insect, both hind legs were removed and the distance between the joints connecting the tibia to the femur and tibia to the tarsus were measured to the nearest 0.001 mm. Measurements were completed using a Leica MZ6 microscope with real-time camera and digital micrometer. Tibial length was recorded as the average of the hind tibiae.

Anthrone Tests. We followed methods of Olson et al. (2000), Lee et al. (2004), Van Handel (1985), and Wyckhuys et al. (2008) in conducting hot anthrone tests, a common method to quantify sugar consumption by parasitoids (Heimpel et al. 2004, Foray et al. 2012). Following storage at -20°C , insects that had been flown in the fed and starved treatments were transferred singly from the 0.5-ml centrifuge tubes to 1.5-ml centrifuge tubes containing $50\ \mu\text{l}$ of 2% sodium sulfate. The insect was crushed using a glass pestle that was sample-specific to avoid contamination. Samples were kept on ice. The pestle was then rinsed into the centrifuge tube using $450\ \mu\text{l}$ of chloroform methanol (1:2), and the resulting solution was mixed using a vortex mixer (referred to as “vortexed”) and centrifuged for 2 min. Centrifugation resulted in the precipitation of a white pellet containing glycogen and a supernatant. The supernatant was pipetted to a 12- by 75-mm glass test tube and further vortexed and the white precipitate was discarded. One hundred microliter of the supernatant was then pipetted into a glass test tube and heated for 2.5 min at 90°C to evaporate the chloroform methanol. This resulted in $\approx 25\ \mu\text{l}$ of solution per tube. After cooling on ice, $975\ \mu\text{l}$ of anthrone reagent was added to each tube to achieve a total volume of $1000\ \mu\text{l}$. Anthrone reagent was also added to three sucrose standards (standard doses of 0, 1, and $5\ \mu\text{g}$) to achieve a total volume of $1000\ \mu\text{l}$. This resulted in $0:1000\ \mu\text{l}$ ($0\ \mu\text{g}$ sucrose), $1:999\ \mu\text{l}$ ($1\ \mu\text{g}$ sucrose), and $5:995\ \mu\text{l}$ ($5\ \mu\text{g}$ sucrose) sucrose: anthrone reagent solutions. The samples and the sucrose standards were vortexed, held at 90°C for 12 min, and cooled in an ice bath. Two hundred microliter from each tube was then added individually into each of two cells (i.e., two replicates per tube) on a 96-well plate. Absorbance was measured at 620 nm using a spectrophotometer. The absorbance of samples both from fed and starved insects was measured simultaneously.

A stock sucrose solution (1 mg/1 ml in a 1:3 ethanol: deionized water solution) was used to prepare the standard dilution series. The standard curve for the dilution series was fit to the 1 and $5\ \mu\text{g}$ standards and through the origin (0,0) after subtracting the mean of the $0\ \mu\text{g}$ absorbance values from all of the samples. The resulting linear equation, $\text{absorbance} = 0.097 \times \mu\text{g sucrose}$ ($F_{1,7} = 41.9$, $P = 0.0003$; $R^2 = 0.86$), was used to convert absorbance to total sugars (μg).

The anthrone reagent was prepared on ice by adding $75\ \text{ml}$ of deionized water into a sterile 1 liter Erlenmeyer flask. A total of $190\ \text{ml}$ of concentrated sulfuric acid (95–97%) was then slowly added into the flask followed by $375\ \text{mg}$ of anthrone. The solution was covered with aluminum foil due to its sensitivity to light, gently mixed until the anthrone had dissolved, and then transferred to an autoclaved 250-ml Corning bottle. The Corning bottle was covered in aluminum foil and stored at 4°C until use.

Release Group. Following data collection, a release group was pooled from data across all experiments. That is, we identified individuals with characteristics matching parasitoids being released in practice across several

states with severe infestations of emerald ash borer (U.S. Department of Agriculture–Animal and Plant Health Inspection Service–Agricultural Research Service–Forest Service [USDA–APHIS–ARS–FS] 2013). We selected females of all sizes that were younger than 10 d old, fed a honey–water solution, and had been housed with males. We used this group to characterize the flight capacities of insects comprising a release cohort.

Statistical Analysis. The relationships between response variables such as total distance flown and flight speed and predictor variables such as age or parasitoid size were analyzed using simple linear regression. Treatments in which predictor variables were categorical, such as feeding status, mating status, or sex, were analyzed using analysis of variance (ANOVA). Graphical inspections of residual plots were used to check assumptions of normality and homoscedasticity of the errors. When necessary, variance-stabilizing square root and log transformations were used. Logistic regression was used to analyze associations between postflight survival status and treatment. All models were fit using a linear mixed-effect model framework, with electronic flight channel as a random effect. All lines of best-fit provided with figures include only variables with slope coefficients that are significantly different from zero. All data analyses were completed using R, and the MASS and lme packages were used for fitting mixed-effects models (Venables and Ripley 2002, Pinheiro et al. 2014, R Core Team 2014).

Results

A total of 308 *T. planipennis* were flown (294 females, 14 males). Females meeting the criteria of the release group (of all sizes, <10 d old, fed, and mated), flew a mean distance of 1.26 ± 0.17 ($\pm\text{SE}$) km (Fig. 1) at average speeds of 0.91 ± 0.03 km/h over a 24-h period ($n_{\text{release}} = 93$). Approximately one-third of the release group flew <100 m and half the release group did not amass total flight distances beyond 500 m, as median distance flown was 0.42 km. The furthest distance flown was 7.16 km in 24 h, achieved by a 3-d-old female. Within the release group, it does not appear that there is a trade-off between flight and postflight survival, as probability of survival after 24 h of attachment to the mill tether arm was not associated with changes in flight distances ($F_{1,36} = 1.47$, $P = 0.23$, $n = 60$).

Total distance flown in 24 h did not decrease with age ($n = 207$; Fig. 2A). Insects at 5 wk of age could fly >5 km, similar to newly eclosed individuals. Mean flight speed decreased significantly with age, though the relationship was not very strong as a one day increase in age was associated with only a 2 m/h decrease in mean flight speed ($n = 207$; Fig. 2B). Older parasitoids were more likely to be deceased at the conclusion of 24 h on the flight mill than younger insects, with 50% postflight mortality predicted at 34–35 d posteclosion ($n = 127$; Fig. 2C).

On an average, parasitoids that were fed a dilute honey–water solution flew a mean of ≈ 2.1 km farther,

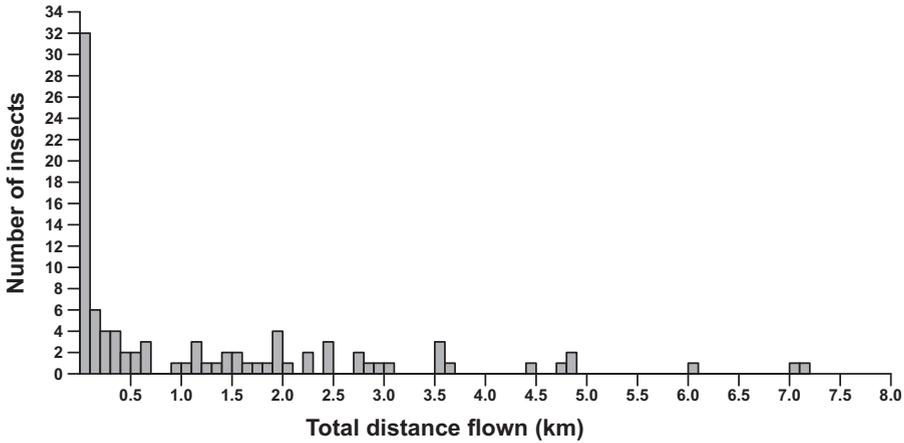


Fig. 1. Frequency histogram of flight distances of female *T. planipennis* that were provided honey and water, mated, and were <10 d old ($n_{\text{release}} = 93$). This group reflects those insects released in practice in biological control programs for emerald ash borer.

or $41\times$ farther, ($F_{1,21} = 32.0, P < 0.0001; n = 45$) and 1.4 times faster ($F_{1,21} = 17.6, P = 0.0004; n = 45$) than parasitoids that were fed only water (Fig. 3A and B). Anthrone tests revealed that fed parasitoids contained almost $10\times$ the amount of total sugars than starved parasitoids did ($F_{1,21} = 10.5, P = 0.0039; n = 45$; Fig. 3C). There was no significant difference in postflight survival status between fed and unfed insects ($F_{1,21} = 1.20, P = 0.29; n = 45$).

Depriving parasitoids of potential mates did not significantly affect flight distance ($F_{1,84} = 0.28, P = 0.60; n = 109$), flight speed ($F_{1,84} = 2.04, P = 0.16; n = 109$), or postflight survival ($F_{1,162} = 0.001, P = 0.97; n = 187$). Female parasitoids flew over $5.8\times$ farther ($F_{1,89} = 14.8, P = 0.0002; n = 113$) and $1.2\times$ faster ($F_{1,89} = 6.33, P = 0.0136; n = 113$) than male parasitoids did (Figs. 4A and B), and there was no significant difference between the postflight survival status of females and males ($F_{1,49} = 1.02, P = 0.32; n = 73$). Female *T. planipennis* had an average of $1.24\times$ longer hind tibiae than males did ($F_{1,17} = 25.3, P = 0.0001; n = 37$). When we tested the relationship between flight parameters and size among females, we found that total distance flown (Fig. 5A) and flight speed (Fig. 5B) were significantly, positively correlated with the mean length of the hind tibiae ($n = 46$).

Discussion

In biological control programs, it is critical to match parasitoids with hosts in both space and time (Hawkins et al. 1993). A long lifespan and ability to forage at advanced ages should relax the requirement for synchronized development of a parasitoid with its host. Our finding that female *T. planipennis* may fly up to 2 km in 24 h on the flight mill at up to 8 wk of age (Fig. 2A) suggests that older parasitoids can be stockpiled for release and should not be considered less viable in terms of their short-term energetics. *T. planipennis* has been shown to survive a median of 6 wk at 25°C and $\approx 65\%$ RH in

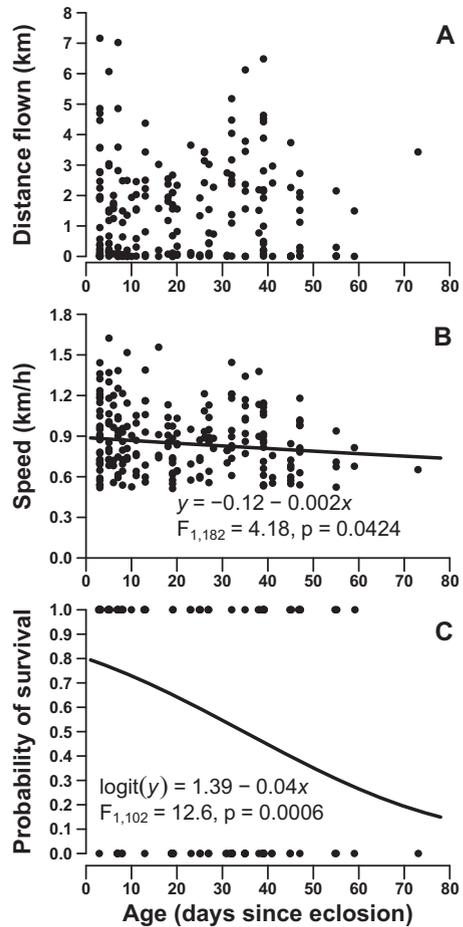


Fig. 2. Relationship of (A) total distance flown over 24 h ($n = 207$), (B) mean flight speed over 24 h ($n = 207$), and (C) survival status at conclusion of a 24-h flight trial with age in days since eclosion ($n = 127$). Lines of best fit are only presented where statistically significant trends exist.

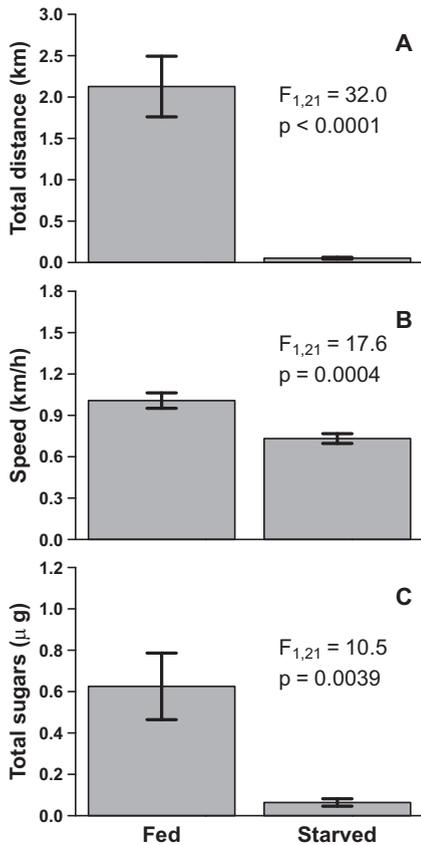


Fig. 3. Comparisons between female *T. planipennis* that were fed a dilute honey-water solution or starved: (A) total distance flown, (B) mean flight speed over a 24-h flight trial, and (C) total sugars as determined using a hot anthrone test ($n = 45$).

laboratory studies (Duan et al. 2011). More critically, there does not appear to be a trade-off between fecundity and longevity for this insect (Duan et al. 2011). Because this parasitoid prefers to oviposit in fourth-instar host larvae (Ulyshen et al. 2010), such longevity may allow *T. planipennis* to await host development to preferred instars following suboptimal emergence or release, while maintaining the energetic capability to locate such hosts. The decrease in postflight survival associated with increased age (Fig. 2C) suggests that older parasitoids are more negatively affected by energy expenditures and desiccation after attachment to the flight mill for 24 h without access to water or carbohydrates.

Indeed, it appears necessary to feed *T. planipennis* a dilute honey-water solution before release to achieve maximum flight capacity. The flight distance of adult parasitoids fed honey with their water was $41\times$ longer than those parasitoids provided only water, indicating that energy stores acquired as larvae are not sufficient to fuel adult flight (Fig. 3A). Honey provisions fueled flight efforts, as postflight

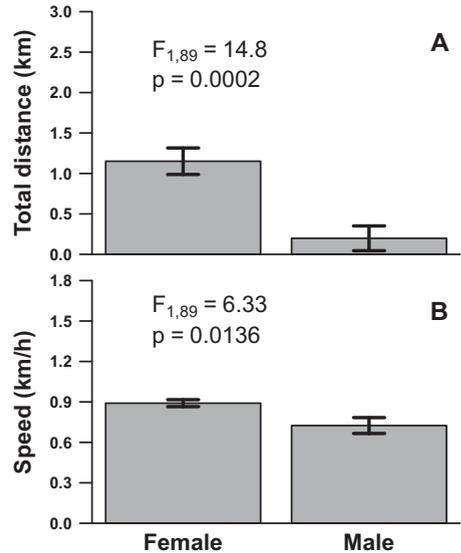


Fig. 4. Comparisons between female and male *T. planipennis*: (A) total distance flown and (B) mean flight speed over a 24-h flight trial ($n = 113$; $F = 99$, $M = 14$).

survival was similar between fed and nonfed treatments and the anthrone tests confirmed higher sugar contents in the insects belonging to honey-provisioned treatments. The role of sugar in the flight capability (Wanner et al. 2006), foraging activity (Wäckers 1994, Lee and Heimpel 2007), fecundity (Winkler et al. 2006, Lee and Heimpel 2008), and survival (Wäckers 2001, Heimpel and

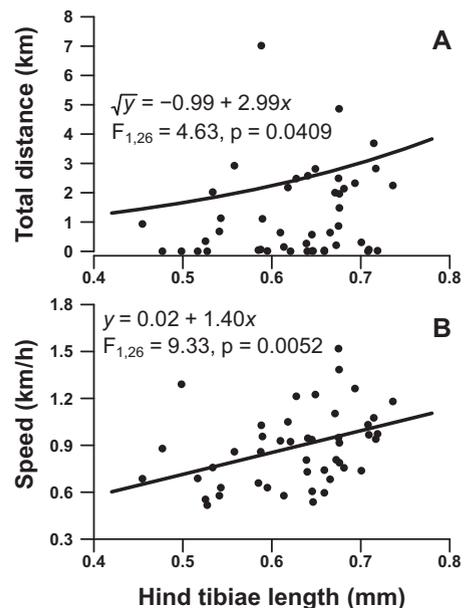


Fig. 5. Relationship of (A) total distance flown and (B) mean flight speed with hind tibiae length (mm) of female *T. planipennis* over a 24-h flight trial ($n = 46$).

Jervis 2005) of hymenopteran parasitoids has been well documented. However, outside of agricultural systems, there is a paucity of information on the role of sugar provisioning in parasitoid–host interactions where the host is an endophytic wood-borer or bark beetle. In two studies of parasitoids of the southern pine beetle *Dendroctonus frontalis* Zimmerman (Coleoptera: Curculionidae), up to three-quarters of parasitoids fed on artificial diet (Eliminate) sprayed onto infested pine trees (Stephen and Browne 2000, Vanlaerhoven et al. 2005). Whereas feeding on Eliminate is associated with increases in longevity of parasitoids of *D. frontalis* (Mathews and Stephen 1997), we do not know how sugar consumption, longevity, fecundity, and flight capacity interact in *T. planipennisi*. Trade-offs between flight and fecundity have been shown for species within orders Hemiptera, Orthoptera, and Lepidoptera (Gunn et al. 1989, Zera and Denno 1997, Zhang et al. 2009b). Sugar provisions for the African armyworm moth, *Spodoptera exempta* Walker (Lepidoptera: Noctuidae), for example, can alleviate a trade-off between flight and fecundity by enabling increased lipid synthesis (Gunn et al. 1989, Zera and Denno 1997).

Males may fly shorter distances than females because of their smaller size, as distances flown by females decreased with size (Fig. 5). However, males may be behaviorally predisposed to fly shorter distances than females. Mating occurs soon after eclosion (Duan et al. 2011). If males and females mate with siblings, as is observed in other *Tetrastichus* spp. (Miller 1966, Hamerski and Hall 1988), or broods emerge synchronously from the same or nearby trees, then males may choose to remain close to their natal tree to maximize the probability of finding a mate. Females may often need to disperse significant distances to find hosts depending on host availability in the natal and adjacent trees, a behavior that could encourage longer flights on the flight mill. No differences in flight capacity were observed between mated and unmated females, but females that have not mated are still able to oviposit. *T. planipennisi* is haplodiploid and produces all-male clutches when eggs are not fertilized (Duan and Oppel 2012). Thus, *T. planipennisi* may not be expected to shift behavior in response to mating.

While flight mills are useful for comparative studies of several variables, limitations of laboratory methods restrict predictive capability in field situations (Kennedy and Booth 1963, Armes and Cooter 1991, Lu et al. 2007). Tethered flight in the laboratory is devoid of variations in host density, chemical cues, natural fluctuations in temperature, wind speed, and precipitation that would affect foraging and dispersal on the landscape, for example. In the native range, we would expect that locating host larvae would require significant foraging efforts by *T. planipennisi* within and between infestations, as emerald ash borers typically exist at low densities in moribund host trees ephemeral in space and time (Wang et al. 2010). In China, high-density infesta-

tions typically only occur where North American species of ash have been planted (Wei et al. 2007). In North America, sporadic distributions of emerald ash borer typical of the insect's native range occur where small, isolated satellite populations have been founded by anthropogenic movement of infested wood (Siegert et al. 2010). Given *T. planipennisi* are very host specific (Liu and Bauer 2006), parasitoid mortality may occur if dispersal away from the release site or natal patch occurs and distances between infestations of emerald ash borer are not traversable. Overall, however, the energetic capabilities and willingness of *T. planipennisi* to initiate flight appear to be comparable with that of its host. Adult emerald ash borers are able to fly up to 7 km/d (mean: $1.3 \pm$ km) over 24 h in similar studies of tethered flight (Taylor et al. 2010). Those results reflect beetles flown in 8-h intervals, separated by times to rest, and provided ash foliage to better represent potential field conditions (Taylor et al. 2010).

Because emerald ash borers are endophagous and tree symptoms such as crown dieback and epicormic shoots are not typically present until trees are heavily infested, the extent and severity of infestations are often challenging to quantify and require destructive branch sampling to determine population presence and density with high certainty (Ryall et al. 2011). Where reliable estimates of the density of emerald ash borers are available, practitioners may fine-tune release procedures for *T. planipennisi*. For example, if populations are highly concentrated at a given release site, releasing a subset of *T. planipennisi* without honey solution might concentrate parasitism locally on the landscape, notwithstanding risk of reduced fecundity or longevity. As further information regarding the establishment and efficacy of *T. planipennisi* becomes available, knowledge of the flight capacity of and optimum flight conditions for *T. planipennisi* may be integrated with spread rates in natural settings to optimize release densities and distances between releases in time and space.

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