

Flight capacity of emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae) and its parasitoid *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), in response to several experimental treatments

A THESIS  
SUBMITTED TO THE FACULTY OF THE  
UNIVERSITY OF MINNESOTA  
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
MASTER OF SCIENCE

Brian H. Aukema (Advisor)

December 2014



## Acknowledgments

I am grateful to my adviser Brian Aukema for agreeing to mentor me. Moving to Minnesota and pursuing my master's thesis has been one of the most formative, challenging, and rewarding experiences of my life. Brian has been an integral part of that experience. I thank Brian for his guidance, encouragement, and most of all, patience. In moments of thesis-panic, I asked many crazy questions. I thank Brian for maintaining his open-door policy despite how challenging it must have been. Having an advisor that cares about their students' as people as much as their development as a scientist was, in hindsight, a necessity for me, and I feel very, very fortunate for landing in Dr. Aukema's lab.

I am also very thankful to the individuals who agreed to be on my advisory committee and their unique contributions to this project and my professional growth. I thank George Heimpel (and his welcoming lab members!) for inviting me to his lab's journal club to discuss, and ultimately introduce me to, parasitoids and biological control. I thank James Forester for challenging me to be a better quantitative scientist. Without his ecological modelling class, I am sure I would still cringe every time I open R. I thank Robert Venette for the helpful discussions on emerald ash borer and experimental design. Most importantly, all of my advisory committee members have set great examples of how careful scientists operate. I would also like to thank Jonathan Lelito, for providing all of the parasitoids and crucial advice on rearing insects. Without insects to fly, there would be no flight mill experiments.

Thanks to my lab mates, Andrea Hefty, Fraser Mckee, Derek Rosenberger, Marissa Streifel and Aubree Wilke for many helpful discussions and for making the lab

an enjoyable place to spend time and attempt some science. Thanks to the Department of Entomology. Through coursework, written prelims, seminar questions, most, if not all, of the faculty members have helped me in some way. Thanks to Felicia Christy, Sally Daly, Rachel Ibes, and Tomi Olayiwola; your daily efforts keep (kept) the department running and are appreciated by all. I thank Mark Abrahamson, Monika Chandler, and Jonathan Osthus of the Minnesota Department of Agriculture who have provided research materials, advice, and many helpful conversations on the management of emerald ash borer in the Twin Cities, MN.

Thanks to Dr. Julie Peterson (University of Nebraska, Lincoln) for help with anthrone testing and to Jonathan Aukema, John Vanstone, and Jeff Cassidy (Western University, London, Ontario, Canada) for their technical expertise in developing the flight mill. Thanks to the Legislative-Citizen Commission on Minnesota Resources, McKnight Land-Grant Professorship funds to BHA, and the College of Food, Agricultural, and Natural Resource Sciences at the University of Minnesota for providing necessary funds to complete this work.

Lastly, I would like to thank my family, Eileen, Bif and Cole. Thanks for the encouragement, and for visiting Minnesota regardless of the season. Clearly, I am here because of you three and your unconditional love and support.

## Thesis Abstract

Emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive beetle native to eastern Asia. *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) is one of three hymenopteran parasitoids currently being introduced into North America as part of a classical biological control program against emerald ash borer. Here, custom-built, computer-monitored flight mills were used to measure the effects of age, feeding status, mating status, sex, and size on flight metrics of *T.*

*planipennisi*. The flight mill was then used to measure flight metrics of emerald ash borer and *T. planipennisi* across a range of temperatures and relative humidity. The relationship between flight energetics, specifically flight distance, with temperature was then integrated with landscape temperatures at ten locations throughout the continental United States to compare relative dispersal capacity for emerald ash borer and *T. planipennisi*.

The goal of this research was to elucidate factors that mediate the flight capacity of both insects and, for *T. planipennisi*, to infer the pre- and post-release conditions that may optimize flight capacity.

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## Thesis Introduction

*Agrilus planipennis* (Coleoptera: Buprestidae), commonly known as emerald ash borer, is a wood-boring beetle that infests ash trees (*Fraxinus* spp.). This insect is native to northeastern China, Japan, Korea, Mongolia, eastern Russia, and Taiwan (Cappaert et al. 2005b). In the spring of 2002, emerald ash borer was discovered near Detroit, MI, USA as the cause of widespread mortality of ash trees (Cappaert et al. 2005b). The initial detection of *A. planipennis* in North America was followed shortly thereafter by another detection in Ontario, Canada (Haack et al. 2002). Dendrochronological analyses showed that the beetle likely became established in Michigan in the early to mid-1990s (Siegert et al. 2007). As of October 2014, *A. planipennis* has now been detected in the following states/provinces: Arkansas, Colorado, Connecticut, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, Wisconsin and Ontario and Quebec, Canada (USDA 2014). It is assumed that *A. planipennis* is capable of infesting all members of the genus *Fraxinus* (Cappaert et al. 2005b, Poland and McCullough 2006, Rebek et al. 2008). Thus, this insect is a threat to maintaining ash as a forest and urban landscape tree throughout North America (Cappaert et al. 2005b).

The continued expansion of the invasive range of emerald ash borer may have severe economic impacts on state agencies, municipalities, and landowners. Ash was a common choice as an urban landscape tree in the 1980s and 1990s, and was a preferred choice to replace elm trees that succumbed to Dutch elm disease (Poland and McCullough 2006). Estimates of the cost of removing dead or moribund, hazardous

urban ash trees range from \$20-60 billion, a figure that does not include the cost of replanting the urban landscape (Cappaert et al. 2005b). The USDA recognizes six species of ash as commercially important (Stewart and Krajicek 1973). Ash lumber, for example, is used for various types of athletic equipment, tool handles, and furniture (Stewart and Krajicek 1973, Poland and McCullough 2006). Distributed across the United States are more than eight billion trees that belong to the genus *Fraxinus* (Poland and McCullough 2006). In 2003, the undiscounted compensatory value of forest ash in the United States was estimated to be \$282.3 billion (Poland and McCullough 2006).

In addition to economic threats, emerald ash borer poses myriad ecological threats to ecosystems that contain ash (Cappaert et al. 2005b, Gandhi and Herms 2010a, b). Ash trees are constituents of 26 different forest cover types across the United States and ash species are widely distributed throughout the upper Midwest and eastern United States (Burns and Honkala 1990, Cappaert et al. 2005b, MacFarlane and Meyer 2005, Gandhi and Herms 2010a). At least 288 species of arthropods are associated with ash (Gandhi and Herms 2010a), 44 of which are classified as being “at high risk” from ash mortality caused by *A. planipennis* (Gandhi and Herms 2010a). Ash trees provide thermal cover for deer and moose as well as potential food for a variety of other mammals such as beavers, rabbits, and porcupines (Cappaert et al. 2005b, Heyd 2005, Poland and McCullough 2006).

Given such economic and ecological threats, large amounts of time and money have been spent on devising management strategies for emerald ash borer. Current efforts aim to slow the spread of *A. planipennis* so that potential economic and ecological impacts are distributed across a larger temporal scale. One of the biggest challenges to

managing emerald ash borer is detecting low density populations (Lyons 2008). No method involving semiochemicals has emerged for effectively detecting low density *A. planipennis* populations. Nonetheless, research on detection methods is ongoing (Francese et al. 2013) and improvements to detection capabilities should aid in population suppression.

The initial response to a new infestation of emerald ash borer typically includes a survey of the surrounding area and a quarantine on the movement of ash firewood and other ash products and materials from the infested county. Managers also use phytosanitation (or sanitation), i.e. the removal of infested host trees, and prophylactic removal of non-infested host materials, to decrease populations of emerald ash borer and the availability of phloem on the landscape. Other options to combat emerald ash borer include several types of insecticides (Herms et al. 2009). However, application of pesticides is a labor-intensive process and is not logistically or economically feasible at the landscape level. Researchers are also working on isolating more tolerant ash trees for future plantings (Meilan et al. 2005), though a resistant or more tolerant cultivar would not ameliorate the current prognosis for standing trees.

One management technique that may become an effective component of an integrated pest management strategy is biological control. After emerald ash borer was discovered in North America, an effort began to establish a classical biological program (Bauer et al. 2008). The program commenced in 2003 with studies on *A. planipennis* and its natural enemies in China (Liu et al. 2003, Bauer et al. 2008). These explorations led to the discovery of multiple Hymenopteran parasitoids, three of which have since been approved as biological control agents and introduced into North America (Liu et al. 2003,

Bauer et al. 2008). Of the three parasitoids, two are gregarious larval parasitoids (*Spathius agrili* Yang (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and one is a solitary egg parasitoid (*Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Bauer et al. 2008). The biological control program for *A. planipennis* is now focused on the widespread introduction and establishment of these biological control agents as well as explorations for new biological control agents. Among naturally occurring biological control agents, woodpeckers have achieved the highest attack rates of any natural enemies, with 9-95% (mean 44%) mortality of *A. planipennis* in some stands of ash in North America (Cappaert et al. 2005a). Research efforts continue to improve the management of emerald ash borer, and future control of this insect will rely on an integrated pest management program employing chemical, cultural, and biological tools.

Research presented in this thesis uses a custom-built flight mill to investigate the flight capacity of the gregarious larval endoparasitoid *Tetrastichus planipennisi* as well as emerald ash borer. In chapter 1, the effect of age, feeding status, mating status, sex, and size on flight capacity of *T. planipennisi* was measured. In chapter 2, the flight capacity of emerald ash borer and *T. planipennisi* was measured in response to changes in laboratory temperatures and relative humidity. The response of flight distance to temperature by both the host and parasitoid are then linked to landscape temperatures to compare potential for flight at several locations throughout the continental United States. At present, Chapter 1 is in press at *Environmental Entomology*. Chapter 2 has been submitted to *BioControl*. There may be redundancy between chapters to maintain the integrity of each as a stand-alone piece of literature.

## **Chapter I**

Factors affecting the flight capacity of *Tetrastichus planipennis* (Hymenoptera: Eulophidae), a classical biological control agent of *Agrilus planipennis* (Coleoptera: Buprestidae)

## 1 Introduction

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 the number of insects available for release is limited. 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, be difficult to sample effectively.

The emerald ash borer *Agrilus planipennis* 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. 2005b, 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 prevents the translocation of nutrients (Poland and McCullough 2006). In China, emerald ash borer is typically associated with stressed ash trees (Wei et al. 2007). In eastern North America, almost all *Fraxinus* spp. appear highly susceptible to insect colonization (Cappaert et al. 2005b, Poland and McCullough 2006, Rebek et al. 2008), though blue ash (*Fraxinus*) exhibits some resistance (Tanis and McCullough 2012). Moreover, native predators and parasitoids are not causing sufficient mortality to reduce populations of emerald ash borer to non-damaging 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. 2005b, Gandhi and Herms 2010b).

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 *Tetrastichus 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).



*Tetrastichus 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 days 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 approximately 27 days (Duan et al. 2011). *Tetrastichus planipennisi* has approximately 4 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). *Tetrastichus 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 employed to study the movement of insects, such as mark-release-recapture with fluorescent dyes, etchings, genetic markers or other non-intrusive 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 paper. 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 relative flight capacity is affected by experimental treatments such as age (Rowley and Graham 1968b, 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, Zhang et al. 2012). Here, we investigate how several variables affect the flight capacity and post-flight 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*.

## **2 Materials and Methods**

### **2.1 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 a) an insect attached to a counterbalanced tether arm, and b) 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 (AWG) (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 grams ( $\pm 0.0006$  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 was 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 greater than 1 second for four consecutive seconds.

## **2.2 *Tetrastichus 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

exited 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 RH on a 16:8 L:D cycle. 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 by 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 one year (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.

### **2.3 *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 seconds 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 3 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) 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.

#### 2.4 *Tetrastichus planipennisi*: experimental treatments

The effects of the following variables on the flight capacity of *T. planipennisi* were investigated: age (number of days post eclosion), mating status, feeding status (honey-water and water), size, and sex (male and female). Parasitoids were typically obtained from groups of 4-6 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 days 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 days 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 ( $22.7^{\circ}\text{C} \pm 0.03\text{ SE}$ ) and humidity ( $20.2\% \text{ RH} \pm 0.43$ ), and were of 24-hour 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-hour flight trial and stored at  $-20^{\circ}\text{C}$  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 days-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 post-flight. Size was measured following

a 24-hour 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.

## **2.5 Anthrone tests**

We followed methods of Olson et al (2000), Lee et al (2004), Van Handel (1985) and Wyckhuys (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 µl 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 µl of chloroform methanol (1:2) and the resulting solution was mixed by using a vortex mixer (referred to as “vortexed”) and centrifuged for 2 minutes. Centrifugation resulted in the precipitation of a white pellet containing glycogen and a supernatant. The supernatant was pipetted to a 12x75 mm glass test tube and further vortexed and the white precipitate was discarded. One hundred µl of the supernatant were then pipetted into a glass test tube and heated for 2.5 min at 90 °C to evaporate the chloroform methanol. This resulted in ~25 µl of solution per tube. After cooling on ice, 975 µl of anthrone reagent were added to each tube to achieve a total volume of 1000 µl. Anthrone reagent was also added to three sucrose standards (standard doses of 0, 1 and 5 µg) to achieve a total volume of 1000 µl. This resulted in 0:1000 µl (0

µg sucrose), 1:999 µl (1 µg sucrose), and 5:995 µl (5 µg sucrose) sucrose: anthrone reagent solutions. The samples and the sucrose standards were vortexed, held at 90 °C for 12 minutes, and cooled in an ice bath. Two-hundred µl from each tube were then added individually into each of two cells (i.e. 2 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 µg standards and through the origin (0,0) after subtracting the mean of the 0 µg absorbance values from all of the samples. The resulting linear equation,  $absorbance = 0.097 * \mu g \text{ sucrose}$  ( $F_{1,7} = 41.9$ ,  $P = 0.0003$ ;  $R^2 = 0.86$ ), was used to convert absorbance to total sugars (µg).

The anthrone reagent was prepared on ice by adding 75 ml of deionized water into a sterile 1 L Erlenmeyer flask. 190 ml of concentrated sulfuric acid (95-97%) was then slowly added into the flask followed by 375 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.

## **2.6 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 (USDA–APHIS/ARS/FS 2013). We selected females of all sizes that were younger



than ten days 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.

## 2.7 *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 post-flight 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).

## 3 **Results**

A total of 308 *T. planipennisi* were flown (294 females, 14 males). Females meeting the criteria of the release group (of all sizes, less than 10 days old, fed, and mated), flew a mean distance of  $1.26 \pm 0.17$  ( $\pm$ SE) km (Figure 1) at average speeds of  $0.91 \pm 0.03$  km/h over a 24-hour period ( $n_{release} = 93$ ). Approximately one-third of the release group flew less than 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 hrs, achieved by a 3-day-old female. Within the release group, it does not appear that there is a tradeoff between flight and post-flight survival, as probability of survival after 24 hrs 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 hrs did not decrease with age ( $n = 207$ ; Figure 2a). Insects at 5 weeks of age could fly more than 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 ( $n = 207$ ; Figure 2b). Older parasitoids were more likely to be deceased at the conclusion of 24 hrs on the flight mill than younger insects, with 50% post-flight mortality predicted at 34-35 days post-eclosion ( $n = 127$ ; Figure 2c).

On average, parasitoids that were fed a dilute honey-water solution flew a mean of approximately 2.1 km farther, or 41× 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 (Figures 3a,b). Anthrone tests revealed that fed parasitoids contained almost 10× the amount of total sugars than starved parasitoids did ( $F_{1,21} = 10.5$ ,  $P = 0.0039$ ;  $n = 45$ ; Figure 3c). There was no significant difference in post-flight 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 post-flight survival ( $F_{1,162} = 0.001$ ,  $P = 0.97$ ;  $n = 187$ ). Female parasitoids flew over 5.8× farther ( $F_{1,89} = 14.8$ ,  $P = 0.0002$ ;  $n = 113$ ) and 1.2× faster ( $F_{1,89} = 6.33$ ,  $P = 0.0136$ ;  $n = 113$ ) than male parasitoids did (Figures 4a,b) and there was no significant difference

between the post-flight survival status of females and males ( $F_{1,49} = 1.02$ ,  $P = 0.32$ ;  $n = 73$ ). Female *T. planipennisi* had an average of 1.24× 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 (Figure 5a) and flight speed (Figure 5b) were significantly, positively correlated with the mean length of the hind tibiae ( $n = 46$ ).

#### **4 Discussion**

In biological control programs, it is critical to match the parasitoids with the host 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 the parasitoid with its host. Our finding that females may fly up to 2 km in 24 hrs on the flight mill at up to 8 weeks of age (Figure 2a) suggests that older parasitoids can be stockpiled for release and should not be considered less viable in terms of their short-term energetics. *Tetrastichus planipennisi* has been shown to survive a median of 6 weeks at 25° and ~65% RH in laboratory studies (Duan et al. 2011). More critically, there does not appear to be a tradeoff between fecundity and longevity for this insect (Duan et al. 2011). Since this parasitoid prefers to oviposit in fourth instar host larvae (Ulyshen et al. 2010), such longevity may allow *T. planipennisi* to await host development to preferred instars following suboptimal emergence or release, while maintaining the energetic capability to locate such hosts. The decrease in post-flight survival associated with increased age (Figure 2c) suggests that older parasitoids are more negatively affected by energy expenditures and desiccation after attachment to the flight mill for 24-hours without access to water or carbohydrates.

Indeed, it appears necessary to feed *T. planipennisi* a dilute honey-water solution prior to release to achieve maximum flight capacity. The flight distance of adult parasitoids fed honey with their water were 41× longer than those parasitoids provided only water, indicating that energy stores acquired as larvae are not sufficient to fuel adult flight (Figure 3a). Honey provisions fueled flight efforts, as post-flight survival was similar between fed and non-fed 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 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). Feeding on Eliminate™ is associated with increases in longevity (Mathews and Stephen 1997), although we do not know how sugar consumption, longevity, fecundity, and flight capacity interact in *T. planipennisi*. Tradeoffs 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 tradeoff between

flight and fecundity by enabling increased lipid synthesis (Gunn et al. 1989, Zera and Denno 1997).

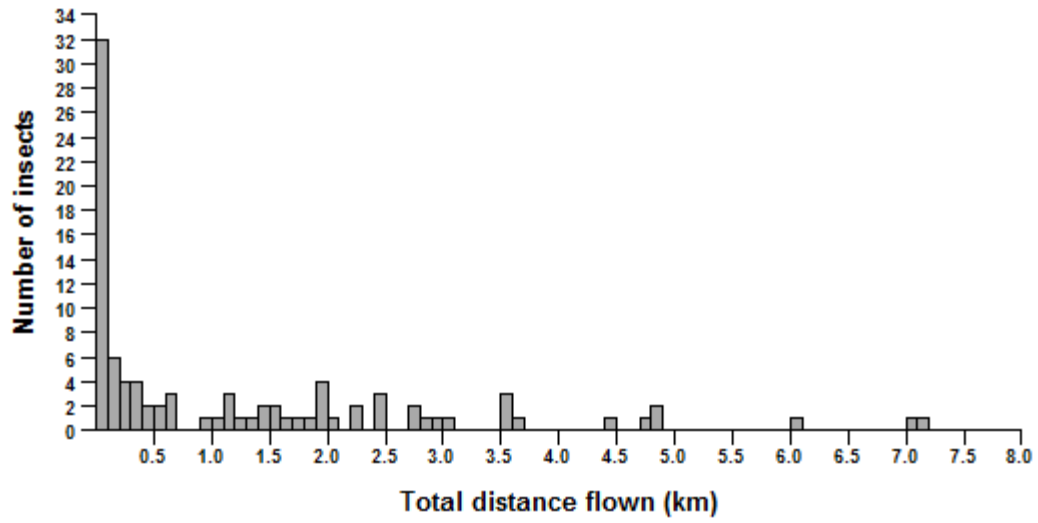
Males may fly shorter distances than females because of their smaller size, as distances flown by females decreased with size (Figure 5). However, males may be behaviorally predisposed to fly shorter distances than females. Mating occurs soon after eclosion (Duan et al. 2011). If males and females mate 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 un-mated females, but females that have not mated are still able to oviposit. *Tetrastichus 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. 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 across their native range in moribund host trees ephemeral in space and time (Wang et al. 2010). High-density infestations typically only occur in China 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 to that of its host. Adult emerald ash borers are able to fly up to 7 km/day (mean:  $1.3 \pm$  km) over 24-hours in similar studies of tethered flight (Taylor et al. 2010). Those results reflect beetles flown in 8-hour intervals, separated by times to rest, and provided ash foliage to better represent potential field conditions (Taylor et al. 2010).

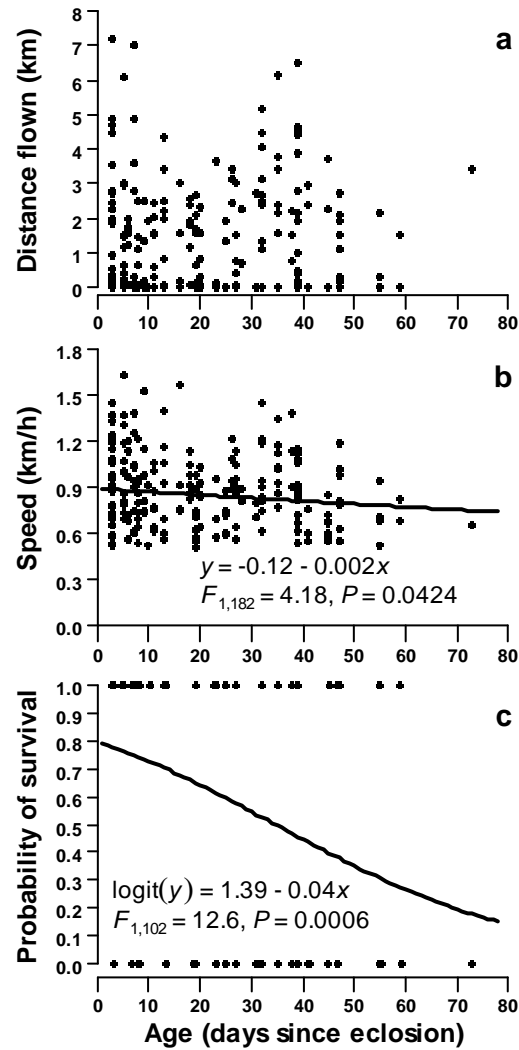
Because emerald ash borers are endophagous and tree symptoms such as crown dieback and epicormic shoots are not typically present until trees are heavily infested, the extent and severity of infestations are often challenging to quantify and require destructive branch sampling to determine population presence and density with high certainty (Ryall et al. 2011). Practitioners may fine-tune release procedures based on the estimated dispersion of emerald ash borer. 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. planipennis* becomes available, knowledge of the flight capacity of and optimum flight conditions for *T. planipennis* may be integrated with spread rates in natural settings to optimize release densities and distances between releases in time and space.

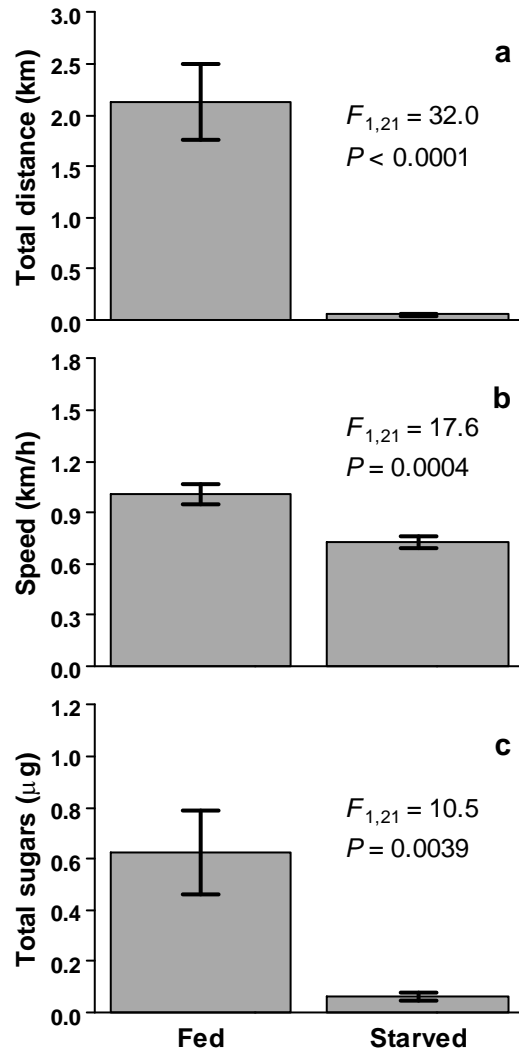


**Figure 1** Frequency histogram of flight distances of female *Tetrastichus planipennisi* that were provided honey and water, mated, and were less than 10 days old ( $n_{release} = 93$ ). This group reflects those insects released in practice in biological control programs for emerald ash borer.

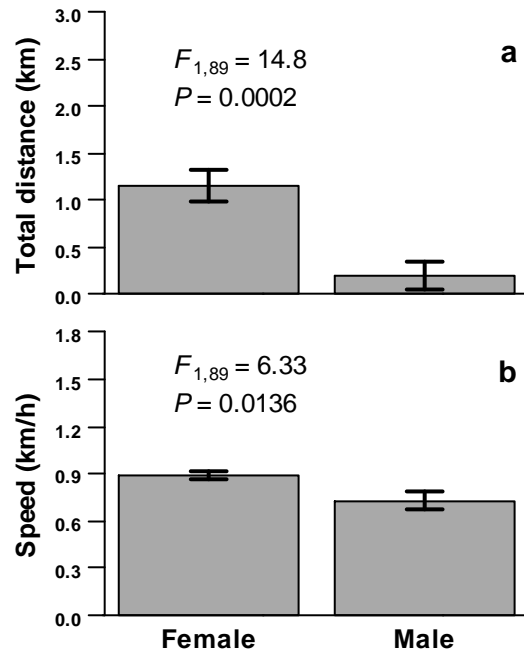




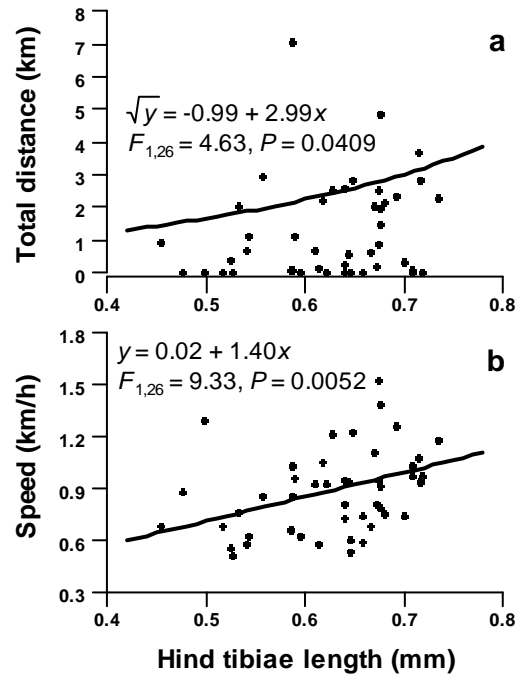
**Figure 2** Relationship of a) total distance flown over 24 hours ( $n = 207$ ) b) mean flight speed over 24 hours ( $n = 207$ ) and c) survival status at conclusion of a 24-hour flight trial with age in days since eclosion ( $n = 127$ ). Lines of best fit are only presented where statistically significant trends exist.



**Figure 3** Comparisons between female *Tetrastichus planipennis* that were fed a dilute honey-water solution or starved: a) total distance flown b) mean flight speed over a 24-hour flight trial c) total sugars as determined using a hot anthrone test ( $n = 45$ ).



**Figure 4** Comparisons between female and male *Tetrastichus planipennisi*: a) total distance flown b) mean flight speed over a 24-hour flight trial ( $n = 113$ ; F = 99, M = 14).



**Figure 5** Relationship of a) total distance flown and b) mean flight speed with hind tibiae length (mm) of female *Tetrastichus planipennis* over a 24-hour flight trial ( $n = 46$ ).

## **Chapter II**

The influence of temperature on the flight capacity of emerald ash borer *Agrilus planipennis* and its parasitoid, *Tetrastichus planipennisi*: Implications to biological control

## 1 Introduction

Classical biological control programs are often established in response to invasive species reaching economically damaging levels. Damaging populations of an invasive species suggest that the abiotic and biotic characteristics of an invaded ecosystem permit sufficient dispersal for finding mates and procuring resources. However, abiotic factors such as climate may mediate the dispersal of imported natural enemies and affect their population dynamics. A meta-analysis by Stiling (1993) studied past classical biological control programs, for example, and implicated suboptimal climatic matching in approximately one half of the failures of the establishment of released natural enemies. Climatic matching is thus critical to success of a classical biological control program. For programs involving an invasive herbivore and insect predators/parasitoids, climatic matching is frequently investigated using insect cold-hardiness testing (Diaz et al. 2008, Hanson et al. 2013), temperature-development comparisons between consumers and resources (Campbell et al. 1974, Diaz et al. 2008), and/or the integration of these components using computer programs (Sutherst and Maywald 1985, Robertson et al. 2008).

Climatic matching often involves elucidating the role of temperature in several metrics of insect performance, including dispersal. Temperature mediates the dispersal of insects in various ways, such as inducing long-term developmental shifts in population morphs (e.g. apterous *vs.* alate) or affecting immediate decisions to initiate and/or continue flight (Taylor 1963, Bale et al. 2002). A decline in flight activity with decreasing temperatures is consistent across several insect taxa (Williams 1940, Forsse et

al. 1992, Briers et al. 2003, Cox et al. 2007). The shape of this decline may differ between pests and natural enemies, however. If disparate dispersal-temperature relationships arise, then changes in regional climates or daily climatic regimes may lead to suboptimal dispersal patterns such as drift of natural enemies into host-free space or accelerated movement by pests away from less motile natural enemies (Heimpel and Asplen 2011). Dispersal patterns that do not maximize encounters between pests and natural enemies may hinder establishment (Heimpel and Asplen 2011). Maximizing probability of establishment may be particularly important in situations when natural enemies are in high demand but of limited supply.

One example where demand for natural enemies is particularly pressing is the biological control program against emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Emerald ash borer, native to Asia, feeds on several species of ash *Fraxinus* spp. (Wei et al. 2007, Wang et al. 2010). This beetle likely became established near Detroit, Michigan, USA and Windsor, Ontario, Canada in the early to mid-1990s but was discovered in 2002 near Detroit causing significant mortality of ash trees (*Fraxinus* spp.) (Haack et al. 2002, Cappaert et al. 2005b, Poland and McCullough 2006, Siegert et al. 2014). *Fraxinus* is a widely distributed genus, particularly in the American Midwest, eastern United States and southern and eastern Canada (MacFarlane and Meyer 2005). Emerald ash borer has yet to infest several areas with known populations of *Fraxinus* spp., but current evidence suggests most North American *Fraxinus* spp. are susceptible (Herms and McCullough 2014). Emerald ash borer poses a threat to several native species of arthropods that use ash as a resource for part or all of their life cycle (Gandhi and Herms 2010a). The cost of removal of infested urban trees

from 2009 to 2019 is estimated at \$10.7 billion (Kovacs et al. 2010, Herms and McCullough 2014). Damage is caused by the larvae, which consume the phloem and cambium, prevent the translocation of nutrients, and eventually stress and kill host trees (Poland and McCullough 2006).

Given the ecological and economic threats posed by emerald ash borer, a classical biological control program against this insect was established in 2003 (Liu et al. 2003, Herms and McCullough 2014). The program began with a survey for natural enemies in China and resulted in the discovery of three species of hymenopteran parasitoids that have since been introduced into North America (Yang et al. 2005, Zhang et al. 2005, Yang et al. 2006, Bauer et al. 2008). *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) is a solitary egg parasitoid, *Spathius agrili* Yang (Hymenoptera: Braconidae) is a gregarious larval ectoparasitoid, and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) is a gregarious larval endoparasitoid (Bauer et al. 2008). *Tetrastichus planipennisi* was first discovered in 2003 in the Jilin and Liaoning Provinces, China (Liu et al. 2003) and has established at release sites in Michigan where it disperses up to 5 km per year (Duan et al. 2013). This parasitoid likely uses host feeding vibrations and chemical cues to locate endophytic host larvae (Ulyshen et al. 2011) and is very host-specific, only attacking emerald ash borer larvae within ash twigs during host specificity testing (Liu and Bauer 2006). Mated females prefer to oviposit in 4th instar host larvae, laying a clutch of approximately 57 eggs per host (Liu et al. 2007, Ulyshen et al. 2010, Duan et al. 2011). *Tetrastichus planipennisi* has multiple generations per year in China and, where established, is likely to have multiple generations per year in North America (Yang et al. 2006, Liu et al. 2007, Duan et al. 2011).



Release guidelines in North America limit releases of *Spathius agrili* to south of 40° latitude to avoid unsuitable winter temperatures. Currently, however, this restriction is the only temperature-related guideline for releasing these three species (USDA–APHIS/ARS/FS 2013). Knowledge of thermal effects on parasitoid performance is limited beyond laboratory studies on cold tolerance of the three parasitoids (Hanson et al. 2013) and the role of temperature in host parasitism rates, development, and survival by *Oobius agrili* (Duan et al. 2014). Here, we use computer-monitored flight mills to elucidate the role of temperature and humidity in the flight patterns of emerald ash borer and its larval endoparasitoid, *T. planipennisi*. Several studies have used flight mills to investigate the flight capacity of native and invasive insects and associated natural enemies in response to changes in temperature and humidity (Taylor 1963, Rowley and Graham 1968a, Heinrich 1971, Zhang et al. 2008, Yu et al. 2009, Liu et al. 2011). Previous studies using flight mills have elucidated the flight capacity of emerald ash borer at room temperature (23-29 °C) and relative humidity (RH) (40-70%) (Taylor et al. 2010), as well as the role of age, feeding status, mating status, sex, and size in the flight capacity of *T. planipennisi* (Fahrner et al. 2014). In the present work, we determine how several flight metrics of both species respond to changes in temperature and humidity, including characterizing the upper and lower temperature thresholds that govern flight initiation and maintenance. We then integrate these results with landscape temperatures at select locations across the United States to elucidate potential changes in flight energetics of both species that may be associated with differences between regional climates. While we recognize the inherent challenges in extrapolating laboratory flights to potential

behavior in the field, our objective is to provide data on flight energetics that may improve future release strategies of *T. planipennisi*.

## **2 Materials and Methods**

### ***2.1 Flight mill and experimental treatments***

We placed six custom-made computer-monitored flight mills (Fahrner et al. 2014) inside two environmental chambers (300 L interior capacity, 24:0 L:D photoperiod) to investigate the role of temperature and humidity in the flight distance, flight speed, number of flights and post-flight mortality of emerald ash borer and *Tetrastichus planipennisi*. Up to six insects of one species were flown at a time. Each insect was flown once for a continuous 24-hour period.

We conducted two sets of experiments for each species. In the first set, the effect of temperature on flight was studied by randomly assigning insects to temperatures between 8-42 °C in increments of 3 °C except for the first increment from 8 to 12 °C that was an increment of 4 °C. At each increment, temperature was held constant at 75% relative humidity. In the second set of experiments, we studied the effect of humidity on flight and held the temperature constant at 27 °C to remain consistent with rearing temperatures (see below). Emerald ash borers were randomly assigned to relative humidities of 50, 65, 75, 85% RH and parasitoids were randomly assigned to relative humidities of 50, 60, 70, 75, 80, 85% RH. Hosts and parasitoids were housed and flown separately. Each insect was attached to the flight mill for a single, continuous 24 hour period, with flight occurring intermittently during that 24 hour flight trial. Following flight, the survival status of insects was determined once by visual examination for one

minute accompanied by tactile stimulation of the legs with one finger. Movements of the legs and wings were judged indicative of survival.

## **2.2 *Emerald ash borer***

All *A. planipennis* were obtained from the USDA-APHIS-PPQ Biocontrol Rearing Facility in Brighton, MI, United States. Upon emergence from logs harvested from the field near Brighton, beetles were separated by sex and provided foliage of green ash (*Fraxinus pennsylvanica*). Within two days of emergence, beetles were shipped to the Forest Entomology Laboratory at the University of Minnesota, Saint Paul, United States. Upon arrival, beetles were held in an environmental chamber (27 °C, ~75% RH 16:8 L:D) in 17 liter plastic rearing containers. Separation by sex was maintained until beetles were attached to the flight mill, as mated females fly significantly farther than their unmated counterparts (Taylor et al. 2010). Beetles were provided new foliage of green ash every 2-3 days as necessary. A 20 cm long, 24 American wire gauge (AWG; diameter: 0.511 mm) wire was used as a tether arm for emerald ash borer. Even numbers of females and males were flown simultaneously except in rare cases where survivorship in rearing containers dictated available sex ratios. In preparation for flight, beetles were randomly selected from their rearing containers and the tether arm was carefully attached to dorsal surface of the pronotum using cyanoacrylate super glue (Loctite® Super Glue Gel; Henkel Corporation). Following successful attachment but before placement onto the flight mill, the tether arm was rotated by hand about the axial needle for 5 seconds to confirm beetles were firmly secured to the tether, able to open their elytra, and able to initiate flight. All beetles were flown within 8 days of eclosion. A total of 132 emerald ash borers were flown: 104 insects for the temperature study and 28 insects for the

humidity study. Treatments were randomized so that temperature and humidity treatments were not confounded with temporal progression of experiments.

### **2.3 *Tetrastichus planipennisi***

*Tetrastichus planipennisi* were also obtained from the USDA-APHIS-PPQ Biocontrol Rearing Facility in MI, USA. Parasitoids used in this study originated from laboratory populations established at the national facility between 2005-2007, and were likely sixty to eighty generations removed from original collection. Parasitoids were shipped as larvae and evenly distributed among glass jars (32 oz.) that were covered with a mesh cloth and placed in an environmental chamber to rear parasitoids to adulthood (27 °C, ~75% RH 16:8 L:D). Adult parasitoids were housed in the same jars in which they eclosed and were provided with a small droplet of honey applied to the mesh cloth cover which was moistened daily with tap water. Attachment of parasitoids to the tether arm followed Fahrner et al. (2014) and insects were randomly assigned to treatments. All parasitoids flown were female, standardized for size by eye, and less than 30 days old (mean of  $11.4 \pm 1.0$  days). We did not separate females from males following eclosion, as flight capacity over 24 hrs is not associated with the presence/absence of males and does not significantly decline as the number of days post-eclosion increases (Fahrner et al. 2014). A total of 114 *T. planipennisi* were flown: 49 insects for the temperature study and 65 insects for the humidity study.

### **2.4 *Defining a flight***

Only insects that flew for three full revolutions within 1 minute of placement onto the flight mill were included in the analyses. It was rare that insects failed to initiate flight during the study and preliminary observations of both the host and parasitoid suggested

that correctly attached, healthy insects initiated flight shortly after attachment to the tether arm. The first five minutes of flight for every insect were removed from the analysis to allow for acclimation from rearing to treatment temperatures. Similar to Fahrner et al. (2014), we discarded data outside of high and low thresholds for speed to preclude rare but spurious recordings of data where an insect aligned the infrared sensor with the edge of a slot on the encoder wheel. For emerald ash borer, we included flights occurring between 0.72–7.5 km/h. This range was developed from preliminary observations of emerald ash borer in tethered flight and reflects previously reported flight speeds of emerald ash borer on a flight mill (Taylor et al. 2010). Flights by emerald ash borer of shorter than ten seconds in duration were also discarded, as small, non-flight wing beats could lead to what we termed “coasting”. For *Tetrastichus planipennis*, flights were only included in analyses if the parasitoid achieved flight speeds between 14–50 cm/s. As before, preliminary observations in the laboratory of this parasitoid in tethered flight were used to develop this range (Fahrner et al. 2014).

## **2.5 Flight Index and landscape temperatures**

Our flight mill experiments allowed us to characterize the relationship between flight capacity and changes in temperature. We used this relationship along with daily maximum, minimum, and mean  $[(\text{maximum temperature} + \text{minimum temperature})/2]$  temperatures over the last 10 years (2003 – 2012) for ten locations throughout the United States to develop a “flight index”. The flight index is a relative scale of estimated flight capacity of emerald ash borer and *T. planipennis* at each city compared to Detroit, MI, United States. The city of Detroit was chosen as the standard due to its significance as the location of initial discovery of emerald ash borer in the United States and the

establishment of *T. planipennisi* in several locations within 130 km of Detroit (Duan et al. 2013). The goal of the flight index was to characterize the suitability of regional climates for dispersal compared to Detroit based on temperature regimes during expected flight periods for both insects. To construct a flight index for a given city, we first calculated daily flight capacities using a smoothing spline (smoothing parameter = 0.5) fit to the relationship between flight distance and temperature (see Figure 6a,b). Temperature was set to mean daily temperature (10 year average) for each day within the flight period (see below) for emerald ash borer and *T. planipennisi*. The flight index was then calculated by summing predicted daily flight distances for each city and dividing each summed total by the summed flight distances for Detroit. Thus, Detroit had a flight index equal to one. All temperature data were obtained from the National Oceanic Atmospheric Administration (NOAA) National Climatic Data Center.

We defined the beginning and end of the flight period for emerald ash borer using growing degree-days (GDD) calculated using the Baskerville and Emin (B-E) method (Baskerville and Emin 1969). All GDD discussed henceforth were calculated using a base temperature of 10 °C. We validated our model for emerald ash borer using reports by Brown-Rytlewski and Wilson (2004), who used the B-E method to calculate GDD associated with the beginning and end dates of the emergence period for emerald ash borer in Ann Arbor, MI, United States in 2003. For comparison, we obtained temperature data from a weather station in Ann Arbor and calculated accumulated GDD for each day in 2003. Brown-Rytlewski and Wilson (2004) reported first emergence from 260-325 GDD (June 5 - 13), which corresponded to May 31 - June 10 using our model and weather data. Similarly, emergence ended at 1056-1157 GDD (August 8 - 16) (Brown-

Rytlewski and Wilson 2004), which corresponded to August 12 - 19 using our model. Given sufficient approximation by our model, and the variation in the beginning of the emergence period between 230-260 GDD (Brown-Rytlewski and Wilson 2004, Poland and McCullough 2006), the flight period was set to begin at 245 GDD. The end of the flight period for emerald ash borer populations in Michigan varies from 1050-1240 GDD (Brown-Rytlewski and Wilson 2004). Emerald ash borer is uni- or semivoltine and thus relevant natural dispersal (i.e. contributing to population spread) will be marked by a single period of emergence and will be limited by the availability of ash foliage for maturation feeding (Poland and McCullough 2006). Thus, the end of the flight period was set at 1145 GDD, the average of the minimum and maximum GDD associated with the end of flight period observed in Michigan (Brown-Rytlewski and Wilson 2004). Days in which the mean temperature was less than 12 °C were not used in calculations of the flight index, as emerald ash borer does not appear to sustain flight below 12°C (see Results).

The beginning of the flight period for *T. planipennisi* was also estimated using GDD calculated with the B-E method. A minimum temperature threshold was used to estimate the conclusion of the flight period. To determine GDD for the start of the flight period, seasonal abundances of parasitoid larvae (within host larvae) during a study in Jingyuetan Forest Park, Jilin Province, China (Latitude: 43.87°, Longitude: 125.35°) (Liu et al. 2007) were used. We obtained temperature data from a weather station in Changchun City, Jilin Province, China (Latitude: 43.9°, Longitude: 125.2°) for 2005 from NOAA and then matched GDD with dates provided by Liu et al. (Liu et al. 2007) to develop GDD-insect presence relationships. In 2005, Liu et al. (2007) confirmed

presence of parasitoids in host larvae from May 15 to November 1. Given that a clutch of *T. planipennisi* requires 7-10 days at 25 °C to kill host larvae, we expected the flight period to begin ~10 days before May 15. This corresponded to emergence at ~90 GDD. Since female *T. planipennisi* eclose with several mature eggs and are able to parasitize hosts shortly after emergence (Duan et al. 2011), we did not include a time-lag between emergence and oviposition.

In contrast to emerald ash borer, the flight period for *T. planipennisi* was based on a minimum temperature threshold and not GDD. *Tetrastichus planipennisi* is multivoltine (Liu et al. 2007), and generations of this insect are likely to develop and emerge continuously until overwintering behaviors are induced by autumnal climatic patterns. Cessation of the flight period of *T. planipennisi* was set as the last day of the season that mean daily temperatures eclipse 15 °C. The 15 °C threshold was chosen because the mean maximum temperature in October 2005 in Jingyuetan Forest Park was 14.9 °C ( $\pm 0.97$ ) (range: 2.1 - 22.6 °C) and parasitoid larvae were present within host larvae in early November (Liu et al. 2007). Those parasitoids likely resulted from a parasitism event sometime in mid-October. *Tetrastichus planipennisi* overwinter as mature larvae in the host gallery (Yang et al. 2006), thus it is likely that the observed broods were still actively feeding (i.e. not overwintering) and that parasitoid larvae did not originate from an ovipositing female earlier in the season than mid-October. Moreover, in tethered flight, parasitoids failed to initiate flight below 15 °C but readily flew and maintained flight at 18 °C (see Results).



## 2.6 *Statistical analysis*

Linear mixed-effects models fit using the nlme package in R (Pinheiro et al. 2014, R Core Team 2014) were used to analyze associations of flight distances, flights speeds, and number of flights with changes in temperature and humidity. A small constant (0.1) was added to each response variable that was log-transformed. Graphical inspections of the residual plots were used to check assumptions of normality and homoscedasticity of the errors. Mixed-effects logistic regressions with canonical links (logit) were fit using the MASS package in R (Venables and Ripley 2002, R Core Team 2014) and used to analyze associations of post-flight survival with temperature and humidity. For models fit using a mixed-effects framework, data acquisition channel of the flight mill was fit as a random effect. Thus, the general framework of our models was as follows: link function( $Y_{ij}$ ) =  $\beta_0 + \beta_1 * \text{predictor}_{ij} + \varepsilon_i + \varepsilon_{ij}$ , where  $\varepsilon_i$  is a normally distributed random error term that can account for any potential variation in recording channel of the flight mill (mean = 0) and  $\varepsilon_{ij}$  similarly represents insect to insect variation on a given mill. Only lines of best-fit that are statistically significant are provided with figures.

## 3 **Results**

### 3.1 *Flight mill*

Across all temperatures and relative humidities, mean flight distance was 1.22 km in 24 hrs of tethered flight (range: 10 m - 6.01 km) and mean flight speed was 3.75 km/h (range: 1.1 – 7.5 km/h) for emerald ash borer. On average, *T. planipennis* flew 1.75 km (range: 1 m-7.1 km) at 1.03 km/h (range: 0.5 – 1.7 km/h). The relationships between flight distances and temperature were similar between species, with decreases in flight capacity occurring towards the high and low ends of the temperature spectra tested.

Maximum predicted flight distance occurred at 27.9 °C and 26.5 °C for emerald ash borer and *Tetrastichus planipennisi*, respectively, where predicted flight distance was 1.04 km and 1.74 km (Figure 6a,b). Flight was minimal (< 500 m) below 12 °C for emerald ash borer. Above 40 °C, however, some insects still flew up to 1 km. *Tetrastichus planipennisi* flew little at air temperatures below 15 °C. Above 39 °C the parasitoid also did not fly far, with distances restricted to < 400 m in 24 hrs.

Responses of mean flight speeds to temperature were markedly different between species. Emerald ash borer's flight speed increased until 35 °C but decreased at temperatures above that threshold (Figure 6c). The flight speed of *T. planipennisi* decreased at temperatures between 8-15 °C but increased from 18-33 °C, exhibiting a plateau at 36 °C (Figure 6d). Emerald ash borer was a much faster flier than its smaller parasitoid. Maximum predicted mean flight speed was 4.7 km/h at 35.1 °C for emerald ash borer and 1.27 km/h at 39.6 °C for *T. planipennisi*. Summaries of regression models presented in Figure 6 can be found in Table 1.

Temperature had a slight but statistically significant, parabolic relationship with the number of flights occurring in a 24-hour period for emerald ash borer ( $\log(y + 0.1) = 0.432 + 0.047*temperature - 0.00043*temperature^2$ ; Figure 7), with the highest rate of flight initiation projected at 26.0 °C. No significant association was found between number of flights and temperature for *T. planipennisi*. The probability that an insect would still be alive after 24 hrs on the flight mill decreased as the temperature increased. Flight trials appeared more stressful for emerald ash borer than *T. planipennisi* as temperatures increased, as 50% mortality was estimated to occur at 22 °C (Figure 8a)

compared to 34 °C, respectively (Figure 8b). Across all temperatures tested, percent mortalities were 66.4% and 28.6% for the host and parasitoid, respectively.

Changes in relative humidity were not associated with significant changes in flight distance for either species. Flight speed, however, did decline with increases in relative humidity for emerald ash borer (Figure 9). An increase from 50 to 85% RH was associated with a decrease of 1.23 km/h in mean flight speed by emerald ash borer. No relationship was found between flight speed and relative humidity for *T. planipennisi*. The total number of flights per insect during a 24-hour flight trial was not associated with changes in relative humidity for emerald ash borer but had a positive, linear relationship with relative humidity for *T. planipennisi* (Figure 10a). This relationship was weak but statistically significant, as an increase from 50 to 85% RH was associated with an increase in ~13 flights per insect over a 24-hour flight trial. Survival probabilities for emerald ash borer were also not affected by changes in relative humidity. For *T. planipennisi*, probability of survival increased with relative humidity (Figure 10b) such that 50% post-flight survival was predicted at a relative humidity of 47% RH. However, experimental humidity ranged from 50-85% RH, and extrapolations beyond the experimental range of humidities tested should be made with caution.

### **3.2 *Flight index and landscape temperatures***

The flight index can be interpreted as relative projected flight capacity of host and parasitoid compared to insects in Detroit based on 10-year averages of daily temperatures. In other words, a location with a flight index of greater than 1.0 would exhibit a climate where an insect would be energetically capable of flying a greater distance than insects near Detroit based on performance on the laboratory flight mill at

various temperatures, projected emergence timing in the field, and average daily temperatures for that location over the past decade. Conversely, insects from a location with a flight index of less than 1.0 would be expected to disperse less distance than a standard reference population in Detroit.

Generally, flight indices were variable across latitudes for emerald ash borer and increased with decreasing latitude for *T. planipennisi* (Table 2). Detroit seems to be a suboptimal, below-average climate for flight capacity for both species. Out of the ten locations studied, only Minneapolis, MN had a lower flight index for emerald ash borer than Detroit. For *Tetrastichus planipennisi*, three locations had a lower flight index than Detroit: Denver, CO, Minneapolis and Seattle, WA. Minneapolis had the lowest flight index for emerald ash borer at 0.99 whereas Seattle had the lowest for *T. planipennisi* at 0.78. The highest flight index was in Seattle (1.32) for emerald ash borer and Phoenix, AZ (1.73) for *T. planipennisi*.

From comparing our laboratory results with 10-year mean temperatures since approximately the time emerald ash borer was discovered in North America, we find that the end of the estimated release period for *T. planipennisi* typically occurs in mid to late October. The earliest average terminal date of seasonal flight was September 28 and occurred in Seattle. The latest average terminal date for seasonal flight was in Phoenix, which appeared suitable for releases of *T. planipennisi* until November 28.

#### **4 Discussion**

Our findings of flights greater than 1 km at ecologically-relevant temperatures (18-36 °C) for both insects suggest that comparable flight capacities may be observed for hosts and parasitoid in natural settings. We do view our extrapolation to field settings

with appropriate caution, however, as tethered flight eliminates behavioral components of dispersal. Insects in our laboratory system, for example, could not respond to host plant cues emanating from the distribution of ash phloem on the landscape as they do in natural settings (Siegert et al. 2010, Mercader et al. 2011). In field settings, most galleries of emerald ash borer occur within 500 m of the preceding year's population, even though emerald ash borer may disperse and oviposit at distances greater than 600 m in a single season (Mercader et al. 2009, Siegert et al. 2010). By comparison, *T. planipennisi* may spread up to 5 km per year, though this distance may be achieved by incremental dispersal of successive generations in a given season (Duan et al. 2013).

Our studies are comparable with other laboratory results, however. Taylor et al. (2010) found that virgin females fly a mean of 820 m in tethered flight at 23-29 °C (16:8 L:D photoperiod). Our projected 24-hour flight distance of 1.04 km for virgin male and female emerald ash borers at 28 °C (temperature at which maximum flight occurred) was similar (Figure 6a). Our insects were flown in complete light, which likely affected flight distance (Taylor et al. 2010). Fahrner et al. (2014) found that mean flight distance for female *T. planipennisi* that were held with males, fed a honey-water solution, and less than 10 days old was 1.26 km at 23 °C, comparable to a predicted flight distance of 1.54 km at 23 °C in the present work (Figure 6b).

Our studies are helpful in examining comparative trends in temperature or humidity. The finding that 50% post-flight mortality occurred at a temperature 12 °C higher for *T. planipennisi* than for emerald ash borer was surprising. *Tetrastichus planipennisi* may exhibit higher adaptation to a wider range of temperatures than its host, as adult stages of the parasitoid are active on the landscape for a longer period of time

(Liu et al. 2007). Larger insects have lower surface area to body mass ratios, however, and are hypothesized to be more resistant to extreme variation in temperature (Chown and Gaston 1999). Emerald ash borers measure approximately 1-1.5 cm in length, whereas female *T. planipennisi* are approximately 4 mm in length (Yang et al. 2006). Increases in the probability of mortality of both insects with temperature suggest that decreases in flight distances at higher temperatures may be due to heat stress and/or desiccation. Indeed, the increase in survival with increasing humidity for *T. planipennisi* suggests decreased rates of desiccation at higher humidity for this insect. In contrast, emerald ash borer may be more sensitive than *T. planipennisi* to heat stress because increases in humidity did not significantly improve survival rates for the host. Insects that are exposed to stressful, high temperatures can alleviate heat stress by producing heat shock proteins (Feder and Hofmann 1999). We are unsure whether insufficient production of heat shock proteins, or simply increased physical susceptibility to desiccation, pre-flight rearing conditions, or diet led to the higher mortality at lower temperatures for the larger host vs. smaller parasitoid. We might expect greater flight activity and flight distances at higher temperatures for both insects, however, when allowed to rest and consume water and/or nutrition in natural settings (Taylor et al. 2010).

The association of spread and dispersal rates of non-native insects with temperature across both short and long temporal scales (Hanks et al. 1998, Lantschner et al. 2014, Tobin et al. 2014) allows us to offer some broad comparisons of regional variation in flight capacity of emerald ash borer and *T. planipennisi* based on regional temperatures (Table 2). Although the flight index does not account for important factors that affect population spread and establishment, such as wind speeds, precipitation

patterns, potential asynchrony between host plants, herbivores and natural enemies (Bale et al. 2002), temperature-induced mortality (Bale et al. 2002), and ash distribution (Mercader et al. 2011), we would expect flight capacity and initiation to respond to local climate regimes as both species arrive in novel locations. *Tetrastichus planipennisi* does not appear to disperse well at temperatures below 15°C, for example (Figure 6b), which may lead to decreased spread rates and/or efficacy in cooler climates. *Tetrastichus planipennisi* prefers to oviposit in 4th instar host larvae and does not appear to detect hosts that have progressed into mature, 4<sup>th</sup> instar (J-shaped) larval or pupal stages (Liu et al. 2007, Ulyshen et al. 2010). If this 15 °C threshold is reached early in the season while host larvae are still developing, then failure to initiate flight may decrease parasitism rates. Further research is needed to determine relationships between temperature and parasitoid efficacy. Moreover, *Fraxinus* spp. are sparsely distributed in the western United States compared to the Midwest and eastern United States (MacFarlane and Meyer 2005). As the introduced ranges of emerald ash borer and the parasitoid expand, the dispersion of ash in different regions may affect dispersal patterns of both insects. Most importantly, dispersal in this system is also driven by anthropogenic transport of firewood (Poland and McCullough 2006). Thus, long-range dispersal via firewood may result in disparate spread rates between host and parasitoid as well as enemy-free space for emerald ash borer if parasitoids are not concomitantly moved or introduced.

As the introduced range of both insects continues to expand, relationships between climate and flight capacity may aid in predicting relative spread rates of both the host and parasitoid based on changes in regional climates. More immediately, managers may be able to maximize or minimize flight capacities of *T. planipennisi* in response to

the dispersion of emerald ash borer on the landscape. Use of weekly temperature forecasts to coordinate releases of this parasitoid at times that maximize dispersal may be preferred if the host infestation spans a large area. Releases at temperatures associated with intermediate flight capacity may be advisable when the host is concentrated on the landscape. However, releases at high temperatures above those that maximize flight capacity may be problematic if the parasitoids do not have access to water or carbohydrates, as flight capacities and survival likely suffer due to desiccation and heat stress. The response of flight capacity to temperature is one of many factors that mediate dispersal by insects, and the role of landscape features (i.e. distribution of host beetles and host plants) and other insect performance - climate relationships in dispersal (discussed above) warrant further investigation. Nonetheless, temperature is an important determinant in the flight activity of both of these species and should be considered when selecting sites and times for release for the parasitoid and in projections of relative spread rates for emerald ash borer and *T. planipennisi*.



**Table 1** Summary of regression models of flight distance and flight speed on temperature for emerald ash borer and *Tetrastichus planipennis* during 24 hour flight trials in the laboratory

<b>Fig. 6</b>	<b>Response</b>	<b>Coefficient<sup>1</sup></b>	<b>Estimate</b>	<b>SE</b>	<b>df</b>	<b><i>t</i></b>	<b><i>p</i></b>
a	EAB flight distance	$\beta_0$	-4.495	0.715	96	-6.29	< 0.0001
		$\beta_1$	0.331	0.057		5.79	< 0.0001
		$\beta_2$	$-6 \times 10^{-3}$	$1 \times 10^{-3}$		-5.61	< 0.0001
b	<i>T.p.</i> flight distance <sup>2</sup>	$\beta_0$	-5.836	1.05	42	-5.58	< 0.0001
		$\beta_1$	0.485	0.089		5.45	< 0.0001
		$\beta_2$	$-9 \times 10^{-3}$	$2 \times 10^{-3}$		-5.36	< 0.0001
c	EAB flight speed <sup>2</sup>	$\beta_0$	-1.139	0.315	96	-3.62	0.0005
		$\beta_1$	0.188	0.025		7.46	< 0.0001
		$\beta_2$	$-3 \times 10^{-3}$	$5 \times 10^{-4}$		-5.75	< 0.0001
d	<i>T.p.</i> flight speed <sup>3</sup>	$\beta_0$	1.833	0.453	41	4.05	0.0002
		$\beta_1$	-0.147	0.064		-2.29	0.0271
		$\beta_2$	$6 \times 10^{-3}$	$3 \times 10^{-3}$		2.31	0.0257
		$\beta_3$	$-8 \times 10^{-5}$	$4 \times 10^{-5}$		-2.10	0.0415

<sup>1</sup> $\beta_0$  represents intercept,  $\beta_1$  is coefficient for temperature,  $\beta_2$  is coefficient temperature<sup>2</sup>,

and  $\beta_3$  is coefficient for temperature<sup>3</sup>

<sup>2</sup>Log transformed

<sup>3</sup>Square root transformed

**Table 2** Flight index, a relative scale of estimated flight capacity, for emerald ash borer and *Tetrastichus planipennisi* based on performance on the laboratory flight mill under varying thermal regimes, projected emergence timing in the field based on growing degree-day models, and mean daily temperatures over the past decade. Flight index is compared to Detroit, MI, United States. Values > 1.0 reflect a greater expected dispersal capacity relative to populations near Detroit, while values < 1.0 reflect a lesser expected dispersal capacity. For derivation of the flight index, please see page 36.

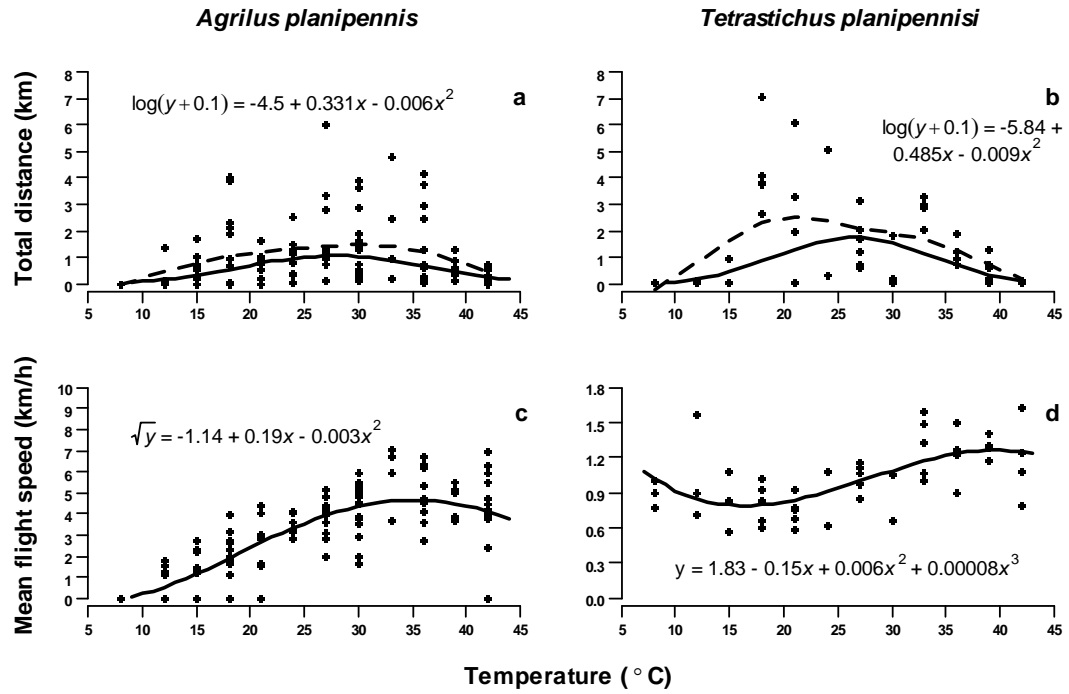
City	State	IATA <sup>1</sup>	Latitude	Longitude	Flight Index		End flight date <sup>4</sup> <i>T.p.</i>
					<i>A. p.</i> <sup>2</sup>	<i>T. p.</i> <sup>3</sup>	
Seattle	WA	SEA	47.45	-122.30	1.32	0.78	Sep 28
Minneapolis	MN	MSP	44.88	-93.22	0.99	0.99	Oct 8
Detroit	MI	DET	42.42	-83.02	1.00	1.00	Oct 9
Boston	MA	BOS	42.37	-71.03	1.02	1.01	Oct 10
Denver	CO	DEN	39.83	-104.66	1.02	0.99	Oct 3
Sacramento	CA	SMF	38.52	-121.50	1.13	1.41	Oct 29
Charlotte	NC	CLT	35.22	-80.93	1.04	1.36	Oct 27
Atlanta	GA	ATL	33.65	-84.43	1.03	1.49	Nov 3
Phoenix	AZ	PHX	33.43	-112.02	1.08	1.73	Nov 28
Dallas	TX	DFW	32.90	-97.03	1.02	1.67	Nov 20

<sup>1</sup>International Air Transport Association airport code

<sup>2</sup>*Agrilus planipennis*

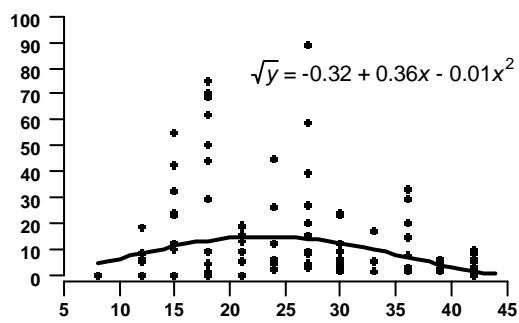
<sup>3</sup>*Tetrastichus planipennisi*

<sup>4</sup>End date reflects expected last day of flight of *T. planipennis* based on seasonal decline of mean daily maximum temperatures below a threshold of 15 °C (Figure 6b).

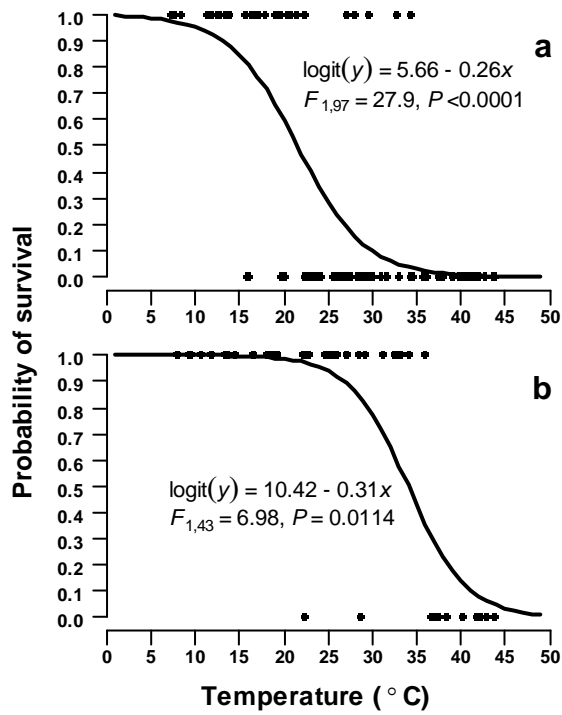


**Figure 6** Relationship of total distance flown of a) *Agrilus planipennis* ( $n = 104$ ) b) *Tetrastichus planipennis* ( $n = 49$ ) and of mean flight speed of c) *Agrilus planipennis* ( $n=104$ ) d) *Tetrastichus planipennis* ( $n = 49$ ) to temperature over a 24-hour flight trial.

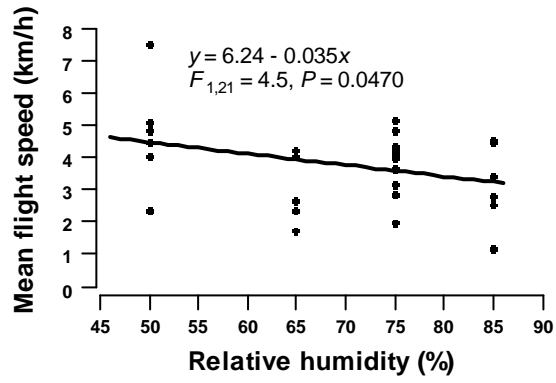
For model coefficients, see Table 1.



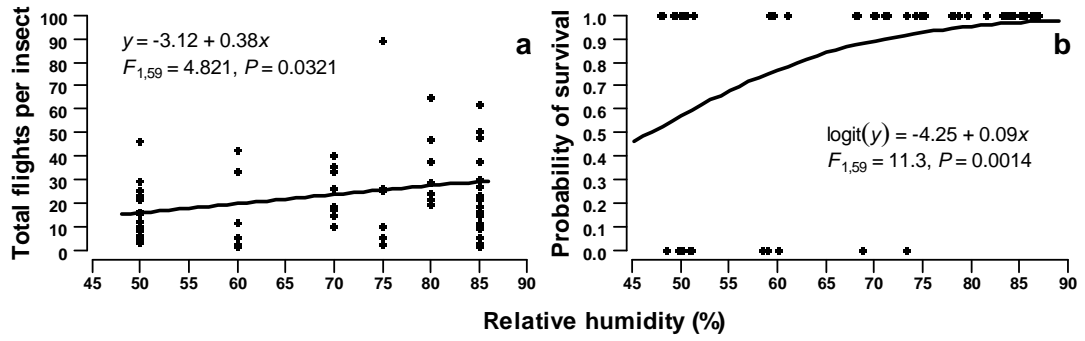
**Figure 7** Relationship of number of flights over a 24-hour flight trial to temperature for *Agrilus planipennis* (n=104).



**Figure 8** Relationship of survival status after a 24-hour flight trial to temperature for a) *Agrilus planipennis* ( $n = 104$ ) b) *Tetrastichus planipennisi* ( $n = 49$ ).



**Figure 9** Relationship of mean flight speed over a 24-hour flight trial to relative humidity for *Agrilus planipennis* ( $n = 28$ ).



**Figure 10** Relationship of a) flights initiated over a 24-hour flight trial and b) survival status after a 24-hour flight trial to relative humidity for *Tetrastichus planipennis* ( $n = 65$ ).



## Thesis conclusions

### Chapter 1

1) ***Tetrastichus planipennis* are able to engage in flights greater than 1 km at 50 days post eclosion.** Given that the median life span of *T. planipennis* in the laboratory is ~ 6 weeks, this parasitoid might remain on the landscape and energetically capable of engaging in long distance flights for several weeks.

2) **Sugar provisions, in the form of a dilute honey-water solution, significantly increase flight distances of *T. planipennis* compared to unfed individuals.** The practice of providing honey to adults of *Tetrastichus planipennis* prior to release should continue. It remains unknown how sugar resources are acquired by this parasitoid in natural settings.

3) **Female *Tetrastichus planipennis* are able to fly farther than their smaller, male counter parts.** Flight distances within female *T. planipennis* are also associated with increases in hind tibiae length.

### Chapter 2

4) **The relationships between flight distances and temperature were similar between both *A. planipennis* and *T. planipennis*, with decreases in flight capacity occurring towards the high and low ends of the temperature spectra tested (8 – 42°C).**

Maximum flight distance occurred at 27.9 °C and 26.5 °C for emerald ash borer and *Tetrastichus planipennis*, respectively, where predicted flight distance was 1.13 km and 1.81 km in 24 hours.

5) **The probability that an insect would still be alive after 24 hrs on the flight mill decreased as the temperature increased for both species.** Flights without rest are more

stressful for emerald ash borer as temperatures increase, as 50% mortality was observed at 22 °C compared to 34 °C for *T. planipennisi*.

6) **Extrapolating flight capacity in the laboratory to thermal regimes in the field, it appears that the potential for flight for both insects decreases with increasing latitude.** Detroit, near where emerald ash borer was discovered in North America, seems to exhibit an intermediate to below-average climate for flight capacity. Out of the ten locations studied, only Denver, CO, Minneapolis, MN and Seattle, WA had a lower flight index for emerald ash borer than Detroit. For *T. planipennisi*, four locations had a lower flight index than Detroit: Boston, MA, Denver, Minneapolis and Seattle.

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