

Effects of Adult Feeding and Overwintering Conditions on Energy Reserves and Flight Performance of Emerald Ash Borer (Coleoptera: Buprestidae)

Dylan A. Tussey,^{1,3,4} Brian H. Aukema,¹ Anthony M. Charvoz,¹ and Robert C. Venette²

¹Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108, ²USDA Forest Service Northern Research Station, 1561 Lindig Street, St. Paul, MN 55108, ³Present Address: Entomology and Nematology Department, University of Florida, Gainesville, FL, and ⁴Corresponding author, e-mail: dtussey@ufl.edu

Subject Editor: Melody Keena

Received 26 September 2017; Editorial decision 1 March 2018

Abstract

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive beetle from Asia, spreads through human-mediated movement and active flight. The effects of adult feeding and overwintering conditions on *A. planipennis* energy reserves (e.g., lipid, glycogen, and sugars) and flight are poorly understood. We conjectured that the potential energetic demands associated with the production of cryoprotectants might affect dispersal capacity and partially explain slower spread of *A. planipennis* in Minnesota than in the other states. Two studies sought to measure the effects of adult feeding on lipid content and flight capacity. Adult *A. planipennis* were fed shamel ash, *Fraxinus uhdei* Wenzig, leaves for 0–20 d after emergence, and half were flown on a custom flight mill for 24 h, before being frozen for comparative lipid analysis with a control group. The second study compared the effects of adult feeding on energy reserves and flight capacity of *A. planipennis* that were originally from St. Paul, Minnesota but overwintered in infested logs placed in Grand Rapids, Minnesota (low winter temperature, –34°C) or St. Paul, Minnesota (–26.3°C). Live adults consumed foliage at a constant rate, but lipid content (percentage of fresh mass) did not change with increases in feeding or flight. Adult glycogen content declined with flight and increased only slightly with feeding. Overwintering location affected survival rates but not energy reserves or flight capacity. These results suggest that the flight capacity of *A. planipennis* is largely determined before emergence, with no differences in energy reserves after cryoprotectant investment.

Key words: *Agrilus planipennis*, dispersal, lipid, glycogen, cold tolerance

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a phloem-feeding beetle from Asia that was first detected in North America in 2002, near Detroit, Michigan, and Windsor, Ontario (Poland and McCullough 2006). This invasive alien insect is a severe pest of native North American ash, *Fraxinus* spp. (Haack et al. 2002, Eyles et al. 2007, Rigsby et al. 2015). The estimated costs of removal and replacement of urban ash trees in North America range from \$5.6 to \$11 billion (Kovacs et al. 2010, Sydner et al. 2011, McKenney et al. 2012).

Larval feeding by *A. planipennis* causes branch dieback and tree death by disrupting the phloem and outer xylem (Cappaert et al. 2005). In a typical univoltine life cycle, late instars excavate pupal chambers in the outer sapwood during autumn in preparation of overwintering. When larval densities are low or where temperatures are cool, the larvae may require 2 yr to complete development, with two overwintering periods. No feeding occurs from the late fourth instar until pupation is complete, so *A. planipennis* larvae must rely

on energy reserves (e.g., glycogen and lipids) obtained over the previous growing season to successfully overwinter and pupate.

Both overwintering and pupation have significant energetic costs. *A. planipennis* is a freeze-intolerant insect; the production of glycerol lowers the supercooling point to avoid the onset of freezing (Crosthwaite et al. 2011). Glycerol and other overwintering compounds, such as trehalose, can be synthesized from glycogen or lipids (Arrese and Soulages 2010). After winter, glycerol may be converted back into glycogen but not at a one-to-one ratio (Chino 1960). Insects must also reach and maintain a minimum viable mass, where fat body lipid content is sufficient to complete pupation (Nijhout 1975, Mirth and Riddiford 2007).

Pupation in spring lasts approximately 30 d before adults emerge and climb into the canopy to feed on foliage (Rodriguez-Saona et al. 2007, Wei et al. 2007, Anulewicz et al. 2008, Wang et al. 2010) for approximately 5–14 d and complete maturation (Wei et al. 2007). Adults have a slight preference for green ash, *Fraxinus pennsylvanica*

Marshall, black ash, *Fraxinus nigra* Marshall, and white ash, *Fraxinus americana* L., over blue ash, *Fraxinus quadrangulata* Michaux, European ash, *Fraxinus excelsior* L., or Manchurian ash, *Fraxinus mandshurica* Ruprecht (Pureswaran and Poland 2009). Adult longevity on white ash foliage (~22 d) is about 1 wk greater than on black ash; green ash is intermediate (Chen and Poland 2010). Total leaf area consumption in 48 h is approximately 22 cm² in choice tests with foliage from green and white ash (Chen and Poland 2010). The effects of maturation feeding on energy reserves in *A. planipennis* are unknown.

Energy reserves are important for mating and dispersal flights of *A. planipennis* (Lelito et al. 2007, Wei et al. 2007). Glycogen is broken down into trehalose for flight muscle energy during short flights, while lipid becomes a flight energy source during prolonged flights (Beenackers et al. 1984, Evenden et al. 2014). Because energy reserves have multiple purposes, a tradeoff may exist between energetic investments in overwintering and flight capacity. For example, monarch butterflies, *Danaus plexippus* (L.) (Lepidoptera: Danaidae), expend large amounts of lipid while overwintering and must replenish energy reserves through feeding before large bouts of flight or reproduction (Chaplin and Wells 1982). Other insects, such as *Manduca sexta* L. (Lepidoptera: Sphingidae), are unable to synthesize lipids as adults so must rely on lipid reserves obtained as larvae (Ziegler 1991).

Since 2002, the range of emerald ash borer in the United States has expanded to 30 states, as of August 2017, with the current northwestern limit in eastern Minnesota (USDA, 2017). Minnesota has nearly 1 billion ash trees; primarily black and green ash (Miles et al. 2016). Emerald ash borer was first detected in the state in 2009, in Ramsey and Hennepin counties. By August 2017, it had spread to 15 counties. As emerald ash borer expands into regions with colder and more prolonged winters, a gradient may exist where *A. planipennis* can survive minimum winter temperatures but experience reduced fitness. The objectives of this study were to compare the energy reserves (i.e., lipid, glycogen, or free sugars) of adult *A. planipennis* that were fed for different periods and flown (or not) on a flight mill. We also compared lipid contents and flight capacity among adults that had overwintered in northern or southern Minnesota. We hypothesized in each study that adult *A. planipennis* feeding would increase energy reserves, and that increased feeding would increase flight capacity. We also hypothesized that colder winter conditions will increase mortality and reduce energy reserves of surviving *A. planipennis*. Reduced energy reserves may ultimately reduce adult flight dispersal capacity and thus slow the spread of *A. planipennis* in colder regions.

Methods

Study Organisms

All *A. planipennis* were collected from naturally infested green ash logs from St. Paul or Minneapolis, Minnesota. The logs were cut from street or park trees where sanitation for management of *A. planipennis* was occurring. All logs were sourced from within a 6.5 km radius. Diameter at breast height (approximately 1.5 m above ground) of all trees used in these studies ranged from 8 to 30 cm.

Seeds of shamel ash, *Fraxinus uhdei* Wenzig, were planted in 15 × 15 × 42 cm plastic pots (Treepot, Corvallis, OR) with mycorrhizae soil mix (Pro-Mix Mycorrhizae, Premier Horticulture Inc., Quakertown, PA) in a greenhouse in January 2015. *F. uhdei* is an evergreen species and is commonly used to feed *A. planipennis* in captivity (Rodriguez-Saona et al. 2006, Crook et al. 2008, 2009). Trees were watered twice weekly with 500 ml each and fertilized

once weekly with 0.25 g of fertilizer composed of 20% by weight for N, P, and K (Plantex Corp., Brampton, Ontario, Canada), throughout the studies. The effects of foliage feeding by adults on lipid reserves and flight were measured in 2015 and again, with modifications, in 2016. The effects of larval overwintering location and adult feeding on energy reserves and flight were measured in 2016.

Effects of Adult Feeding on Lipid Reserves and Flight

In January 2015, 12 green ash trees were cut into logs approximately 60 cm in length and left uncovered, outside our research facility in St. Paul, Minnesota for the remainder of winter. In April 2015, before insect emergence, logs were retrieved from outdoors and randomly sorted into two batches. The logs of one batch were immediately placed in cardboard emergence tubes fitted with glass jars as per the study by Fahrner et al. (2015). Emerged adults were collected daily. A second batch of logs was placed into a walk-in cooler kept at 4°C to delay larval development until use. After 28 d, without any adult emergence, logs from the first batch were replaced by the second batch (on July 3, 2015) and more adults were collected. A total of 216 logs were used in 2015.

Feeding Treatments

In 2015, emerged *A. planipennis* were sexed, weighed, and assigned a feeding treatment (0, 4, 8, 12, 16, or 20 d) and flight designation (no flight or 24 h on flight mill) using a stratified random design at time of emergence. Individual *A. planipennis* were placed in 475-ml plastic containers that were modified to have a 10 cm² hole covered with aluminum mesh for ventilation and a 7.62 cm length floral tube (Royal Imports, Brooklyn, NY) inserted through the side to hold leaflets. Adults in containers were kept on a lab bench at ~23°C and a photoperiod of 16:8 (L:D) h. Mature terminal leaflets of shamel ash were cut and rinsed with deionized water, and petioles were placed in floral tubes to keep leaflets from desiccating during feeding trials. Beetles were allowed to feed ad libitum for the duration of their feeding treatment. Leaflets were replaced when ~50% of the leaf tissue was consumed or if leaves appeared desiccated. All leaves were imaged with a flatbed scanner after removal, and the surface area consumed was estimated using the polygon area measurement tool in ImageJ software (Abràmoff et al. 2004). Beetles not designated for flight treatments (below) were weighed and immediately frozen at -80°C until lipid analysis was performed.

Flight Treatments

Custom computer-monitored flight mills, previously used and described by Fahrner et al. (2014) and Kees et al. (2017), were used to measure the flight capacity of adult *A. planipennis*. Flight tether arms were constructed with 20-cm long copper 108 American wire gauge (diameter ~0.171 mm), with a No. 1 insect pin serving as a central axis, resulting in an arm radius of 10 cm. After insects fed for a prescribed period, the pronotum was attached to one end of the tether arm using cyanoacrylate super glue (Loctite Super Glue Gel; Henkel Corporation) (Machial et al. 2012). All beetles were checked by rotating the flight mill arm in an upright position by hand until their elytra opened. This operation ensured that the tether placement did not interfere with flight initiation. Beetles were placed on flight mills and allowed to fly for 24 h in continuous light (Fahrner et al. 2015). No food, water, or perches were provided. Each flight mill was fitted with an infrared (IR) sensor that recorded all sensitive movements of the tether arm. To account for rare but spurious movements of the tether arm, due to air currents or accidental

bumps during assay initiation, bouts of flight were defined by the following parameters: flight speeds between 0.72 and 7.5 km h⁻¹, durations >5 s, and a minimum of three revolutions. After flight, beetles were removed from the flight mill arm by gently prying them from the dried glue. All beetles were reweighed and frozen at -80°C to preserve tissues until lipids were analyzed.

Lipid Extraction Using Petroleum Ether

In 2015, lipids were extracted with petroleum ether per [McKee and Aukema \(2015\)](#). Before lipid extraction, all beetles were placed in individual wells of a 24-well plate. Beetles were placed in a drying oven for 24 h at 60°C. Dry weights were recorded to the nearest 0.01 mg from all beetles within 1 h of removal from the drying oven to minimize rehydration from ambient humidity. Lipid extraction was performed using 300 ml of petroleum ether circulating through an extraction column and condenser with a round-bottomed flask heated to 45°C. Individual beetles were placed in modified 0.5-ml microcentrifuge tubes, allowing for petroleum ether to flow into and drain out of each tube during extraction. Paper labels with laser-printed numbers were inserted in each tube to identify beetles. Extractions ran with two flushes of the extractor column per hour for a total of 16 h; preliminary analyses indicated that lipid loss from *A. planipennis* stabilized by 16 h of extraction. Beetles were again dried for 24 h at 60°C before being weighed a final time. Lipid weight was determined by subtracting the post-lipid-extraction dry weight from the initial dry weight. For each treatment combination, 8–25 beetles were tested for a total of 158 beetles in this study.

This experiment was repeated in 2016 with the following adjustments. Beetles were reared from a batch of 90 logs. In the previous experiment, we noted increased mortality in the 20-d treatment, likely due to a cessation of feeding sometime after 16 d, so feeding durations were limited to 0, 2, 4, 8, or 12 d. In addition, to emulate the starvation that occurs with the flight treatment in the control group, beetles that were not placed on the flight mill remained in their container for 24 h, without access to food or water, rather than being immediately frozen. For each treatment combination, between 8 and 15 beetles were tested for a total of 96 beetles in this study.

Effects of Winter Temperatures and Feeding on Energy Reserves

In October 2015, green ash (*F. pennsylvanica*, $n = 30$) that were naturally infested with *A. planipennis* were removed from St Paul, Minnesota and brought to the University of Minnesota's St Paul campus. Each main stem was given an identification number before being cut into 60-cm sections. Cut ends were sealed with paraffin wax to reduce desiccation. The logs were stored outside until use.

Two locations in Minnesota were selected to determine the effects of overwintering temperatures on energy reserves, nutrient levels, and flight capacity of *A. planipennis*: St. Paul (44.988792, -93.179854), where below-bark temperatures were expected to be > -30°C, and Grand Rapids (47.247798, -93.495491) where below bark temperatures were expected to be < -30°C. Such low-temperature extremes have been predicted to cause significant mortality to *A. planipennis* ([Venette and Abrahamson 2010](#), [DeSantis et al. 2013](#)).

On 8 January 2016, 70 logs were selected in a stratified random design (by tree) and assigned to one of two groups (35 logs/group). One group was transported to Grand Rapids, where logs were secured with eyebolts and chains and kept in a gated, locked area, to comply with quarantine regulations. Logs were stored from 8 January to 3 March 2016, through the coldest part of winter. The

other group was kept outside our research laboratory in St. Paul for the duration of the study. Both sets of logs were placed upright on wood pallets to keep them above the snowline. Hobo Pro v2, 2 ext temp recorders (Onset Computer Corp., Bourne, MA) were placed on the north and south faces of both pallets. One probe was inserted into the phloem of a log to record below-bark temperatures. The other probe was inserted into a white PVC 3-Gang electrical box to record ambient temperatures for the duration of the experiment.

All logs were brought indoors on 8 March 2016 and placed in cardboard-rearing tubes to allow adult beetles to emerge naturally. Adult beetles were collected daily from 14 April to 9 May 2016. As adult beetles emerged, each was sexed, weighed, and assigned a feeding (0, 4, 8, or 16 d) and flight treatment (no flight or 24 h on flight mill) by using a stratified random design with the random sample function without replacement in R software. This approach ensured that each feeding by flight combination included individuals that emerged on similar dates. For each treatment combination, energy reserves (specifically, lipid, glycogen, and sugars) were measured on 11–23 beetles for a total of 134 beetles in this study. A subsample of logs from both overwintering locations were peeled 4 wk after the last adult *A. planipennis* emerged to count the number of dead larvae, pupae, and adult *A. planipennis*. Mortality was measured by calculating the percentage of dead beetles from the total beetles recovered from each log.

Analysis of Energy Reserves Using Colorimetric Assays

Before analysis, the elytra, wings, and legs were removed from each beetle to aid in processing. Analysis of energy reserves was performed on eight individual beetles at a time. Each beetle was placed in a 1.5-ml microcentrifuge tube and vortexed with 1 ml of deionized water to rinse off external debris. The water was removed, and beetles were crushed in 100 µl of sodium sulfate with a plastic pestle. A solution of chloroform-methanol (1:2 by volume, total volume 450 µl) was added to each vial, washing the pestle in the process. All vials were centrifuged at 1,400 × *g* for 5 min. Supernatant was removed and placed in a glass test tube (13 × 100 mm) for use in lipid and sugar assays. The precipitate was retained for analysis of glycogen.

Standard Curves

Known standards of lipid, glycogen, and sucrose were prepared per [Van Handel \(1985a,b\)](#). Lipid standards were prepared with soybean oil in concentrations of 0, 25, 50, and 100–700 (in increments of 100) µg/ml, with chloroform as the solvent, and placed in clean test tubes. Glycogen standards were prepared with concentrations of 0, 1, 5, 10, 25, 50, 75, and 100 µg/ml, and stored in 1.5-ml microcentrifuge vials until use. Sucrose standards were prepared with concentrations of 0, 1, 5, and 10–50 (in increments of 10) µg/ml, and placed in clean glass test tubes. Fresh standards were made with each trial. Standards were processed as described below to produce a colorimetric change. Light absorbance was measured at predetermined frequencies (stated below) with a spectrophotometer (SpectraCount BS10000, Packard Instrument Company). Standard curves to relate the concentration of each compound to absorbance were created using linear or second order polynomial regressions.

Lipid Assay

Lipids were analyzed using methods modified from [Van Handel \(1985\)](#). From each standard or supernatant, 200 µl was transferred to a clean test tube and heated to 90°C until all liquid evaporated. Each tube was cooled to ambient temperature and 200 µl of sulfuric acid was added. The samples and standards were reheated to 90°C

for 10 min. Test tubes were cooled to ~20°C and filled to a total of 5 ml with vanillin reagent. The solution was allowed to react for 10 min before being plated. Two 200- μ l aliquots from each sample and standard were placed into wells of a 96-well microwell plate. Absorbance at 490 nm was measured. The absorbance readings for two replicates were averaged to estimate the lipid content. The final measurement of lipid was multiplied by 2.75 μ l to account for dilution throughout the assay, yielding a total mass estimate in μ g.

Glycogen Assay

Glycogen was analyzed per Olson et al. (2000). To each microcentrifuge tube containing the precipitate, 1 ml of anthrone reagent was added. Tubes were vortexed for 30 s to dissolve the precipitate and centrifuged at $1,200 \times g$ for 2 min to separate out remaining solids. Half of this solution (500 μ l) was transferred to a new tube, along with an additional 500 μ l of anthrone reagent. Standards were filled to a total of 1 ml with anthrone reagent. The samples and standards were heated at 90°C for 8 min. Two 200- μ l aliquots of each sample and standard were placed into wells of a 96-well microwell plate. Absorbance at 620 nm was measured. The absorbance readings for the two replicates were averaged to estimate the lipid content. The final measurement of glycogen was multiplied by 2.0 μ l to account for dilution throughout the assay, yielding a total mass estimate in μ g.

Sugars Assay

We followed the procedures of Olsen et al. (2000) to measure concentrations of disaccharides using a hot anthrone method. A 100- μ l aliquot of supernatant was transferred to a clean test tube (as previously described) and heated for 4.5 min. Test tubes were placed on ice to cool before 750 μ l of anthrone reagent was added. Vials containing standards were filled to a total of 1 ml with anthrone reagent. The samples and standards were heated at 90°C for 12 min. Two replicates of 200- μ l solution from each sample and standard were placed into wells of a 96-well microwell plate. Absorbance at 620 nm was measured. The absorbance readings for the two replicates were averaged to estimate the disaccharide content. The final measurement of sugars was multiplied by 5.5 μ l to account for dilution throughout the assay, yielding a total mass estimate in μ g.

Statistics

All statistical analyses were conducted with R software (ver.3.3.1). Linear regression models were used to relate leaf area consumption to adult age to estimate feeding rate. Linear regression was used to relate leaf area consumption to change in mass and lipid reserves. Only non-flown beetles were tested for the effects of feeding on lipid reserves to prevent confounding effects of flight. Lipid was expressed as percent of fresh mass because a strong correlation existed between lipid mass and fresh weight. Linear regression was used to relate beetle mass to flight distance. Analysis of variance (ANOVA) was used to compare the effects of adult age and sex on flight metrics (i.e., number of bouts, distance, flight time, and velocity). A two-sample *t*-test was performed to compare lipid reserves of flown versus nonflown beetles.

To test for differences in low temperatures, a data set of the difference of daily low temperatures was compiled. A Durbin-Watson test was used to verify that autocorrelation in the time series, which can bias inferential tests, was successfully removed by thinning the data by removing every other day. A one-sample *t*-test was performed to compare the mean temperature differences between the two sites ($\alpha = 0.05$). An ANOVA was used to compare mortality between sites.

Linear regressions were used to relate leaf area consumption with lipid, glycogen, and sugar reserves among nonflown beetles. ANOVAs were performed to compare glycogen and sugar reserves of

flown versus nonflown beetles. Linear regression was used to relate remaining glycogen content of flown beetles to total flight distance.

Results

Feeding Over Time

At emergence, female and male beetles had a mean mass of 36.56 mg (± 0.71 SEM) and 24.90 mg (± 0.75), respectively. In 2015, beetles consumed leaf tissue at a rate of 1.42 cm²/d ($F_{1,137} = 413.92$, $P < 0.0001$). Beetles would occasionally consume over 20 cm² after a week of feeding. The feeding rate was similar in 2016 (Fig. 1A and B). The mass of males that fed exhibited 5% higher body mass increases than females, irrespective of adult age in 2015 ($F_{1,123} = 9.18$, $P = 0.0033$). However, there were no significant differences in mass change between sexes in 2016. Body mass changed with adult age, with an initial mass gain in the first 8 d, followed by a slight decline in mass (Fig. 1C, D). However, these patterns were not significantly correlated with leaf area consumption ($F_{1,137} = 0.53$, $P = 0.47$). For both years, water content was ~65% of fresh mass of adult beetle at emergence, with water content dropping below 60% by 8 d of adult age.

Effects of Feeding on Lipid

Beetle lipid reserves were not affected by leaf area consumption ($F_{1,67} = 0.02$, $P = 0.88$) in 2015 or in 2016. (Fig. 2). Lipid (% of fresh mass) did not change significantly with adult age ($F_{1,95} = 1.78$, $P = 0.19$). These results were similar in 2016. The mean lipid content for beetles was 2.32% ($\pm 0.16\%$ SEM) in 2015 and 1.66% ($\pm 0.14\%$) in 2016.

Flight Tests

Flight distance increased significantly with the age of unmated, adult beetles in 2015, with total flight distance increasing by 47 m/d (Fig. 3A). A similar result was obtained in 2016 with flight distance increasing by 91 m/d (Fig. 3B). The mean distance flown was 0.54 (± 0.13) km in 2015 and 0.80 (± 0.18) km/d in 2016. Table 1A and B shows flight performance metrics of beetles, based on adult age and sex, in 2015 and 2016. No significant differences between sexes were found for either year. Velocity increased with adult age in 2015, but this trend was not apparent in 2016.

Effects of Winter Temperatures and Feeding on Energy Reserves

Winter Temperatures and Mortality

Below bark temperatures experienced by *A. planipennis* larvae were significantly colder in Grand Rapids, Minnesota than in St Paul, Minnesota ($F_{1,27} = 62.09$, $P < 0.0001$). Temperatures dropped below -30°C four times in Grand Rapids, while temperatures in St Paul remained above -30°C. The lowest below bark temperatures recorded for the Grand Rapids and St Paul were -34°C and -26.3°C, respectively (Fig. 4). Overwintering mortality was significantly greater in Grand Rapids, about 50%, compared to St. Paul, which was approximately 20% ($F_{1,8} = 28.84$, $P = 0.0006$, $n = 10$). Totals of 34 and 90 adults were collected from logs from Grand Rapids and St Paul, respectively. However, overwintering location had no significant impacts on emergence mass, feeding rate, energy reserves, or flight performance. These nonsignificant differences are not reported.

Effects of Feeding on Energy Reserves

Beetle lipid reserves were not affected by leaf area consumption ($F_{1,46} = 1.03$, $P = 0.31$) (Fig. 5A). The average lipid content was

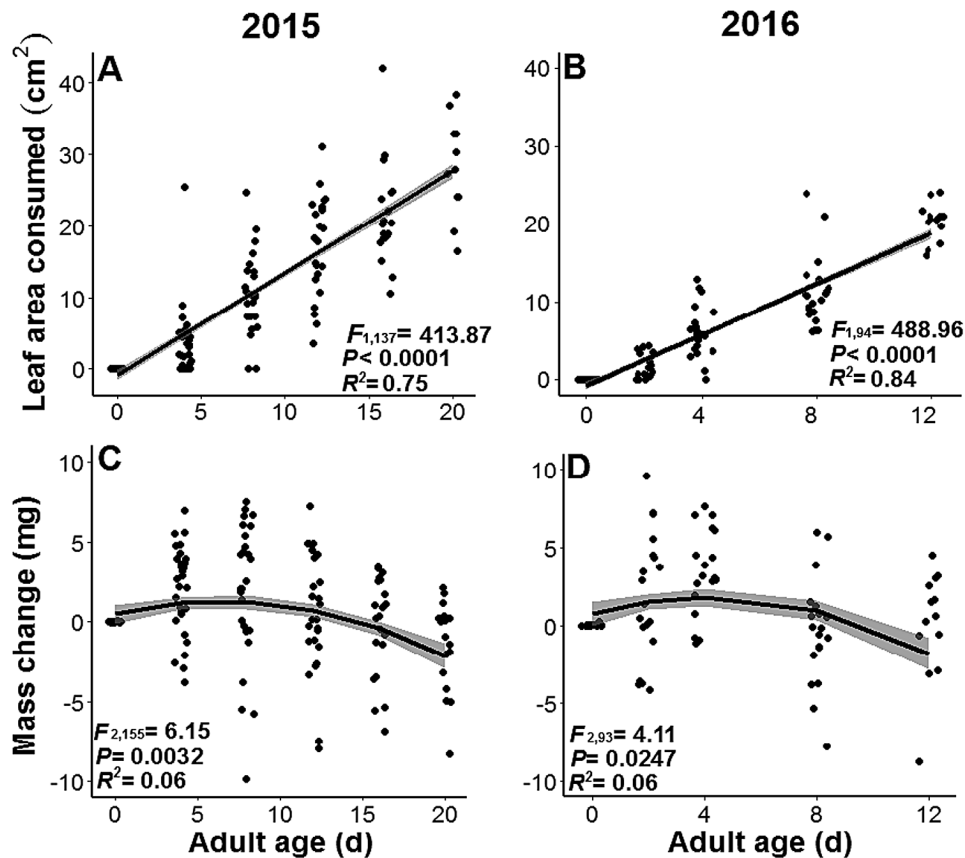


Fig. 1. Relationship of leaf area consumed (A, B) and mass change (C, D) of *Agrilus planipennis* to adult age in 2015 (left) and 2016 (right). Adult age is closely related to leaf area consumed. Gray ribbons associated with regression lines represent standard errors.

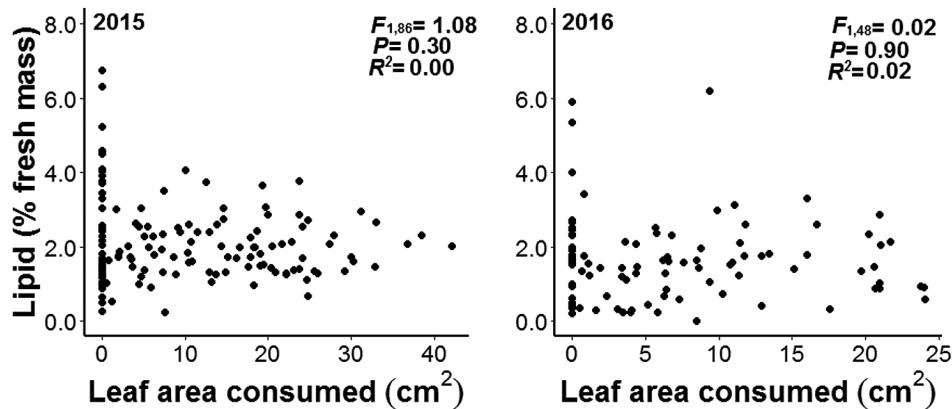


Fig. 2. Lipid content versus leaf area consumed by *Agrilus planipennis* adults in 2015 (left) and 2016 (right). Gray ribbons associated with regression lines represent standard errors.

3.13% of fresh weight ($\pm 0.13\%$). Glycogen content did not increase significantly with leaf area consumption ($F_{1,46} = 0.86$, $P = 0.36$) (Fig. 5B). Total sugars increased significantly with leaf area consumption ($F_{1,46} = 5.39$, $P = 0.0248$) at a rate of $0.003 \mu\text{g}/\text{cm}^2$ (Fig. 5C).

Effects of Flight on Energy Reserves

Beetle mass at the time of flight was a strong predictor of flight distance, along with adult age ($F_{2,55} = 11.48$, $P < 0.0001$). Heavier and older beetles that were allowed to feed ad libitum flew farther on average. Both glycogen ($F_{1,122} = 4.41$, $P = 0.0394$) and sugar ($F_{1,109} = 2.10$, $P = 0.0382$) content (in mg) were significantly lower

in flown versus nonflown beetles, with flown beetles having 0.01 mg less glycogen and 0.04 mg less sugars. Glycogen percent (of fresh mass) decreased with increasing flight distances at a rate of $0.024\%/ \text{km}$ ($F_{1,53} = 5.45$, $P = 0.0233$) (Fig. 6). Table 1C shows flight performance metrics of beetles, based on adult age and sex. No significant differences in flight metrics were found between sexes.

General Flight Observations

Across all studies, 143 of the 155 *A. planipennis* that were placed on flight mills initiated flight. Only 71% of unfed individuals initiated flight at least once during 24 h flight trials ($n = 48$), while 93% of

fed individuals initiated flight at least once ($n = 107$), a difference that was significant ($\log\text{-link}(y) = 0.89 + 1.72x$, $Z = 3.56$, $P < 0.0001$, $n = 155$). The mean flight distance for all unmated adults for both studies in 2015 and 2016 was 0.54 ± 0.08 km (range: 0.002–5.58 km) within 24 h under constant light.

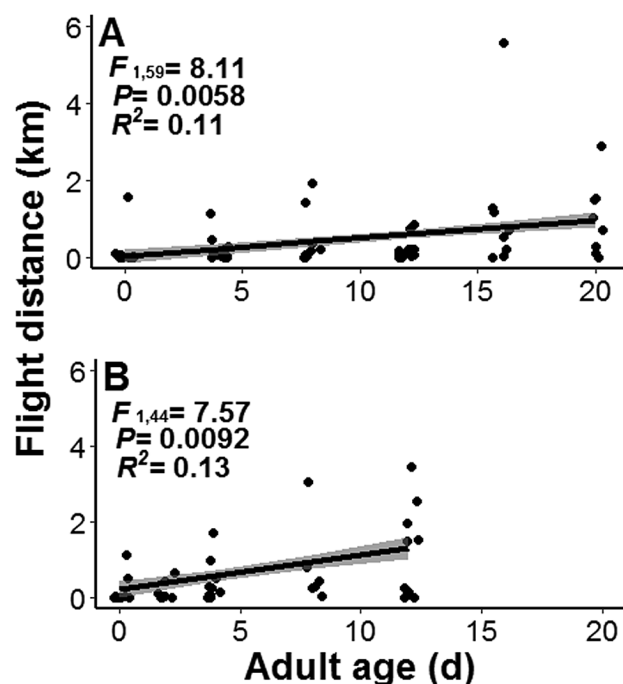


Fig. 3. Total flight distance of *Agrilus planipennis* after 24 h on a flight mill in 2015 (A) and 2016 (B), versus adult age of *Agrilus planipennis*. Gray ribbons associated with regression lines represent standard error.

Discussion

Nearly all *A. planipennis* adults that were allowed to feed on leaf tissue ate vigorously (Fig. 1A and B). However, feeding did not significantly affect mass or lipid content (Fig. 1C and D, Fig. 2). Although feeding did significantly increase sugars (Fig. 5C), and to a lesser extent glycogen, though not significant (Fig. 5B), the increases were slight and unlikely to substantially increase flight potential. It appears more likely that adult feeding serves to maintain water content. It was incidentally noticed that beetles that did not feed would desiccate and die within 2 d.

To our knowledge, this study was the first to measure flight of *A. planipennis* from Minnesota. Beetles in this study flew 0.54 km in 24 h on average. All beetles were unmated, and no significant differences were found between sexes. These distances were shorter than the mean flight distance of 1.22 km in 24 h (maximum: 6.01 km) that Fahrner et al. (2015) reported for unmated *A. planipennis* from Michigan. The present study used the same flight mills as Fahrner et al. (2015), but with more lenient criteria to define a flight, yet still recorded substantially shorter flight distances. The difference in flight capacity measured by Fahrner et al. (2015) and the current study might be explained by potential differences in host quality, overwintering conditions, adult-handling procedures, or flight conditions (i.e., temperature and relative humidity while on the mill), among others. Taylor et al. (2010) measured flight of *A. planipennis* from southeastern Michigan. Raw data from the study indicates that unmated males had a mean mass of 32.1 (± 1.6) mg and flew 0.53 km (± 0.18 ; maximum: 3.29 km) in 24 h of constant light; unmated females had a mean mass of 33.5 mg (± 1.8) and flew 0.33 km (± 0.07 ; maximum: 0.92 km) under the same conditions (R.A.J. Taylor, Ohio State University, personal communication), differences in the design, and operation of the flight mills. Beetle mass at the time of flight was a strong predictor of flight distance. Any factor that affects adult size may affect flight potential.

Table 1. Mean number of bouts^a, mean total distance flown, mean total time in flight, and mean velocity during flight bouts ($\pm$ SE) of *Agrilus planipennis* by adult age and sex after 24 h on flight mill experiments conducted in 2015 (A) and 2016 (B–C^b)

Adult Age	No. Bouts		Distance (km)		Time (min)		Velocity (kph)		<i>n</i>	
	F	M	F	M	F	M	F	M	F	M
A 0	10.2 $\pm$ 1.4	1.0 $\pm$ 0.0	0.354 $\pm$ 0.302	0.003 $\pm$ 0.002	9.6 $\pm$ 7.7	0.1 $\pm$ 0.1	1.50 $\pm$ 0.08a	1.81 $\pm$ 0.91	5	2
4	41.8 $\pm$ 17.6	5.5 $\pm$ 1.5	0.364 $\pm$ 0.166	0.056 $\pm$ 0.017	10.0 $\pm$ 4.1	1.4 $\pm$ 0.3	1.46 $\pm$ 0.08a	1.72 $\pm$ 0.32	6	4
8	28.8 $\pm$ 7.7	17.0 $\pm$ 0.0	0.544 $\pm$ 0.352	0.745 $\pm$ 0.695	12.4 $\pm$ 6.6	18.4 $\pm$ 15.7	1.89 $\pm$ 0.44ab	1.32 $\pm$ 0.30	4	2
12	22.6 $\pm$ 7.5	16.5 $\pm$ 6.5	0.244 $\pm$ 0.086	0.064 $\pm$ 0.006	5.9 $\pm$ 1.5	17.4 $\pm$ 15.4	1.88 $\pm$ 0.22ab	1.49 $\pm$ 0.01	11	2
16	39.3 $\pm$ 30.1	9.5 $\pm$ 1.5	1.500 $\pm$ 0.841	0.269 $\pm$ 0.266	28.7 $\pm$ 20.3	5.6 $\pm$ 4.2	2.18 $\pm$ 0.22ab	1.52 $\pm$ 0.36	6	2
20	30.5 $\pm$ 11.4	46.0 $\pm$ 40.0	0.860 $\pm$ 0.247	1.457 $\pm$ 1.443	13.5 $\pm$ 5.0	31.5 $\pm$ 30.8	2.52 $\pm$ 0.40b	1.83 $\pm$ 0.02	6	2
	$F_{6,54} = d1.22$ $P = 0.313$		$F_{6,54} = 2.37$ $P = 0.041$		$F_{6,54} = 1.11$ $P = 0.372$		$F_{6,54} = 3.98$ $P = 0.002$			
B 0	12.3 $\pm$ 10.3	11.5 $\pm$ 8.5	0.202 $\pm$ 0.164	0.292 $\pm$ 0.282	4.1 $\pm$ 3.3	4.9 $\pm$ 4.3	2.67 $\pm$ 0.36	2.32 $\pm$ 0.62	3	4
2	63.0 $\pm$ 54.0	6.3 $\pm$ 2.3	0.242 $\pm$ 0.209	0.211 $\pm$ 0.153	11.3 $\pm$ 9.8	3.7 $\pm$ 2.7	1.28 $\pm$ 0.05	2.36 $\pm$ 0.41	2	4
4	45.5 $\pm$ 9.4	30.5 $\pm$ 8.6	1.095 $\pm$ 0.542	1.089 $\pm$ 0.797	23.5 $\pm$ 10.3	20.9 $\pm$ 15.7	2.14 $\pm$ 0.25	2.42 $\pm$ 0.27	6	4
8	74.7 $\pm$ 53.7	13.3 $\pm$ 7.4	1.255 $\pm$ 0.898	0.377 $\pm$ 0.221	30.3 $\pm$ 23.0	8.8 $\pm$ 5.8	2.42 $\pm$ 0.16	2.15 $\pm$ 0.21	3	3
12	38.6 $\pm$ 15.8	29.0 $\pm$ 22.0	1.112 $\pm$ 0.386	1.810 $\pm$ 1.658	18.7 $\pm$ 8.0	35.0 $\pm$ 32.6	2.54 $\pm$ 0.22	3.21 $\pm$ 0.39	7	2
	$F_{5,40} = 2.14$ $P = 0.082$		$F_{5,40} = 1.83$ $P = 0.128$		$F_{5,40} = 1.49$ $P = 0.214$		$F_{5,40} = 1.58$ $P = 0.188$			
C† 0	21.2 $\pm$ 11.0	5.8 $\pm$ 2.4	0.177 $\pm$ 0.052a	0.133 $\pm$ 0.065	5.0 $\pm$ 2.0a	2.8 $\pm$ 0.9	2.46 $\pm$ 0.31	1.92 $\pm$ 0.41	9	4
4	29.3 $\pm$ 17.3	39.8 $\pm$ 12.3	0.255 $\pm$ 0.147a	0.172 $\pm$ 0.050	6.8 $\pm$ 3.2ab	7.4 $\pm$ 2.1	1.94 $\pm$ 0.20	1.33 $\pm$ 0.07	6	6
8	22.2 $\pm$ 5.6	20.7 $\pm$ 8.0	0.522 $\pm$ 0.243ab	0.170 $\pm$ 0.066	10.5 $\pm$ 4.2ab	4.1 $\pm$ 1.4	2.09 $\pm$ 0.20	2.05 $\pm$ 0.28	9	6
16	26.5 $\pm$ 10.9	23.7 $\pm$ 8.8	0.793 $\pm$ 0.288b	0.513 $\pm$ 0.206	15.3 $\pm$ 5.4b	10.8 $\pm$ 4.1	2.59 $\pm$ 0.42	2.26 $\pm$ 0.35	6	5
	$F_{4,53} = 1.33$ $P = 0.271$		$F_{4,53} = 4.09$ $P = 0.006$		$F_{4,53} = 3.32$ $P = 0.017$		$F_{4,53} = 2.68$ $P = 0.041$			

ANOVA results describe the effect of age on flight; the effect of sex was not significant. If significant differences among ages were present, letters beside values of females denote similar values.

^aBouts are defined by the following parameters: flight speeds between 0.72 and 7.5 km h⁻¹, durations >5 s, and a minimum of three revolutions.

^bThis experiment used insects from a population in St. Paul, Minnesota, some of which were stored in Grand Rapids, Minnesota, for the winter.

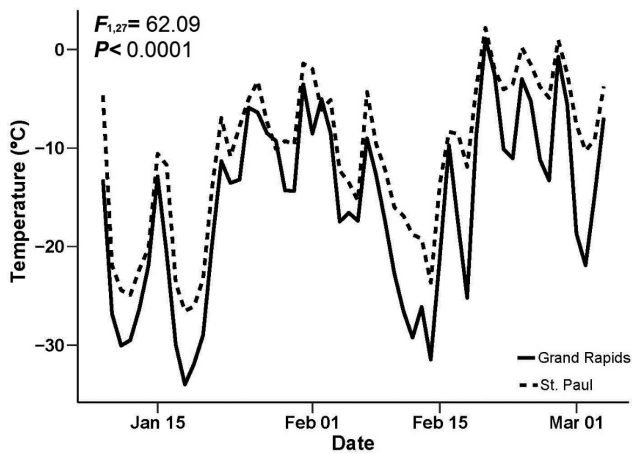


Fig. 4. Daily low temperature recorded below bark of *Fraxinus pennsylvanica* logs stored in Grand Rapids, Minnesota or St. Paul, Minnesota from 8 January 2016 to 3 March 2016.

All *A. planipennis* collected had greater masses of lipid reserves than glycogen or sugars. This may indicate that lipid is preserved through winter, while glycogen and sugars are used for production of overwintering compounds. In this study, adult feeding did not have any impact on lipid reserves. This finding may indicate that *A. planipennis* does not synthesize lipids as adults.

Similarly, lipid content did not significantly decrease with flight, suggesting that lipids may be more important for purposes other than flight fuel, such as reproduction. Lipid is often important in ovarian development and egg production (Anderbrant 1988, Briegel 1990, Zera and Larsen 2001, Sisterson et al. 2015).

One potential reason for similar energy reserve levels for both groups could be that the temperature threshold for metabolization of energy reserves in *A. planipennis* is relatively high. Hayakawa and Chino (1981), for example, found a temperature-dependent relationship for glycogen conversion into overwintering compounds in pupae of *Samia cynthia* Drury (Lepidoptera: Saturniidae), where glycogen is rapidly converted into trehalose once exposed to low temperatures. Furthermore, the production of trehalose was initiated through enzymes that activate at 2°C and maintain at colder temperatures (Hayakawa and Chino 1982). *A. planipennis* may have a similar response, where mechanisms for cold tolerance activate in relatively warm temperatures and do not differ at colder temperatures.

Although we did not detect any sublethal effects of cold with respect to dispersal capacity, potential impacts may exist. Because of increased mortality in Grand Rapids, Minnesota, our sample size of survivors may have been too small to detect significant differences in energy reserves or flight capacity between overwintering locations. It is also possible that our treatment locations were not different enough to elicit differences in sublethal effects. *A. planipennis* may be at its physiological limit in St. Paul, so colder temperatures in Grand Rapids only result in increased mortality. We also did not test for any fecundity differences. Tradeoffs have been noted between energy expenditure during overwintering and fecundity as adults in goldenrod gall flies, for example (Irwin and Lee 2000).

It is interesting to note that the mean glycogen level was nearly identical for both groups of beetles. If *A. planipennis* invest in antifreeze compounds only at the onset of winter, similar glycogen levels between groups would be expected, as all beetles in this study

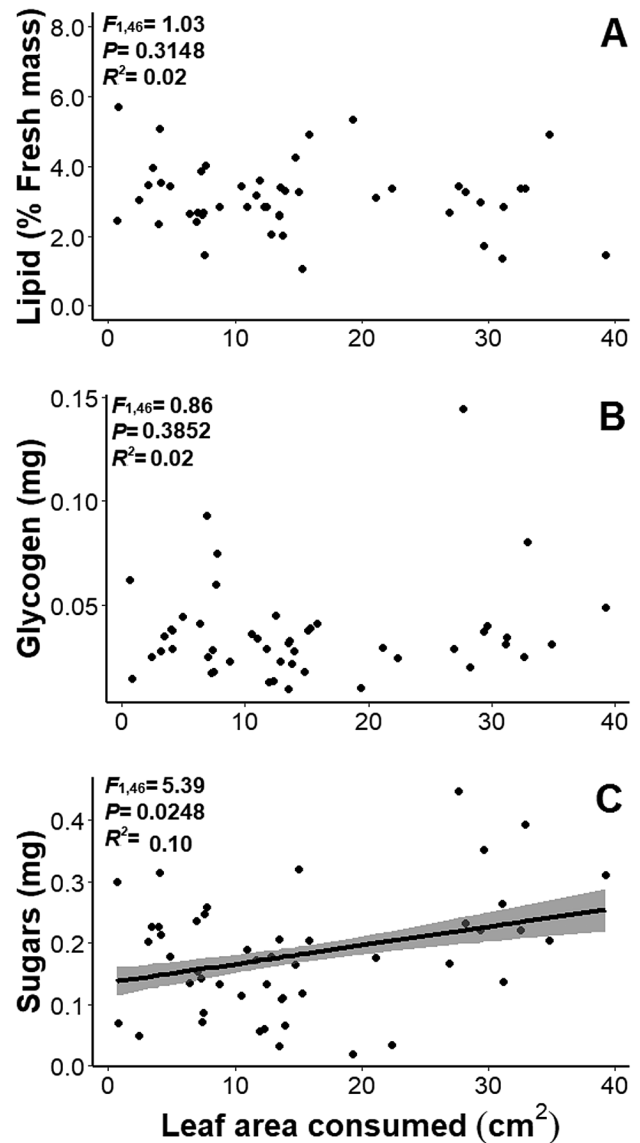


Fig. 5. Lipid (A), glycogen (B), and sugars (C) of adult *Agrilus planipennis* versus leaf area consumed. Gray ribbons associated with regression lines represent standard errors.

originated from the same population and began overwintering in St. Paul, Minnesota. Crosthwaite et al. (2011) found that *A. planipennis* produce large amounts of glycerol in November and the levels remain stable from December to March. It has also been found that *A. planipennis* begins to catabolize glycerol at the onset of warmer temperatures and that this process is irreversible (Sobek-Swant et al. 2012).

Our findings suggest that colder winter temperatures are not directly reducing the flight capacity of *A. planipennis* overwintering at the current northwestern margin of this insect's expanding range in North America. Although mortality was significantly higher among *A. planipennis* larvae that had overwintered in Grand Rapids, Minnesota than in St. Paul, Minnesota, feeding and flight performance among emerged adults was not different. As such, *A. planipennis* do not appear to be utilizing more energy reserves in colder locations.

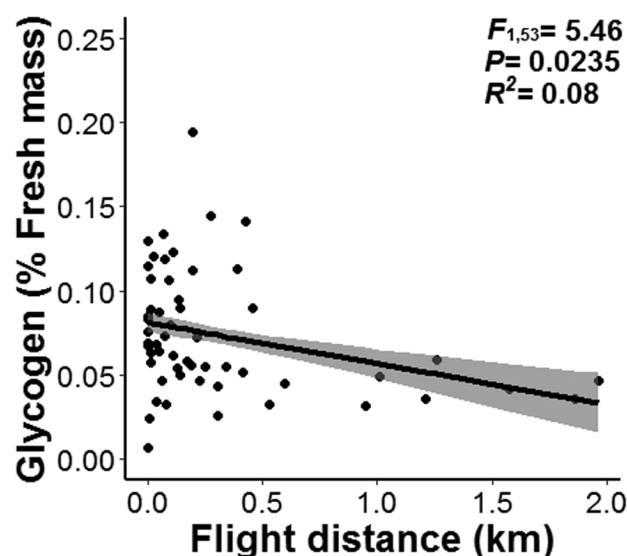


Fig. 6. *Agrilus planipennis* glycogen content (% of fresh mass) versus total distance flown (km) in 24 h on flight mill in 2016.

Acknowledgments

Funding for this study was provided by the Environment and Natural Resources Trust Fund project M.L. 2013, Chp. 52, Sec.2, Subd. 06cB as recommended by the Legislative-Citizen Commission on Minnesota Resources. We are grateful to George E. Heimpel (University of Minnesota, USA) for his time, lab space, and equipment to help with anthrone and vanillin testing, Paul Castillo (USDA, Forest Service, Northern Research Station, St. Paul, MN) for his help in the field and greenhouses, and to Rachel Coyle (City of St. Paul, MN) for her assistance in obtaining infested wood. This MS thesis research of DT benefited from the constructive comments of Gary Johnson (University of Minnesota).

References Cited

- Abbramoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with imageJ. *Biophotonics Int.* 11: 36–41.
- Anderbrant, O. 1988. Survival of parent and brood adult bark beetles, *Ips typographus*, in relation to size, lipid content and re-emergence or emergence day. *Physiol. Entomol.* 13: 121–129.
- Anulewicz, A. C., D. G. McCullough, D. L. Cappaert, and T. M. Poland. 2008. Host range of the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in North America: results of multiple-choice field experiments. *Environ. Entomol.* 37: 230–241.
- Arrese, E. L., and J. L. Soulages. 2010. Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* 55: 207–225.
- Beenakkers, A. M. T., D. J. Van der Horst, and W. J. A. Van Marrewijk. 1984. Insect flight muscle metabolism. *Insect Biochem.* 14: 243–260.
- Briegleb, H. 1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. *J. Insect Physiol.* 36: 165–172.
- Cappaert, D., D. G. McCullough, T. M. Poland, and N. W. Siegert. 2005. Emerald ash borer in North America: a research and regulatory challenge. *Am. Entomol.* 51: 152–165.
- Chaplin, S. B., and P. H. Wells. 1982. Energy reserves and metabolic expenditures of monarch butterflies overwintering in southern California. *Ecol. Entomol.* 7: 249–256.
- Chen, Y., and T. M. Poland. 2010. Nutritional and defensive chemistry of three North American Ash Species: possible roles in host performance and preference by Emerald Ash Borer adults. *Gt. Lakes Entomol.* 43: 20–33.
- Chino, H. 1960. Enzymatic pathways in the formation of sorbitol and glycerol in the diapausing egg of the silkworm, *Bombyx mori*—I. On the polyol dehydrogenases. *J. Insect Physiol.* 5: 1–15.
- Crook, D. J., A. Khirman, J. A. Francese, I. Fraser, T. M. Poland, A. J. Sawyer, and V. C. Mastro. 2008. Development of a host-based semiochemical lure for trapping emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae). *Environ. Entomol.* 37: 356–365.
- Crook, D., J. Francese, K. Zylstra, I. Fraser, A. Sawyer, D. Bartels, D. Lance, and V. Mastro. 2009. Laboratory and field response of the emerald ash borer (Coleoptera: Buprestidae), to selected regions of the electromagnetic spectrum. *J. Econ. Entomol.* 102: 2160–2169.
- Crothwaite, J. C., S. Sobek, D. B. Lyons, M. A. Bernards, and B. J. Sinclair. 2011. The overwintering physiology of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). *J. Insect Physiol.* 57: 166–73.
- DeSantis, R. D., W. K. Moser, D. D. Gormanson, M. G. Bartlett, and B. Vermunt. 2013. Effects of climate on emerald ash borer mortality and the potential for ash survival in North America. *Agric. For. Meteorol.* 178–179: 120–128.
- Evenden, M. L., C. M. Whitehouse, and J. Sykes. 2014. Factors influencing flight capacity of the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae). *Environ. Entomol.* 43: 1–10.
- Eyles, A., W. Jones, K. Riedl, D. Cipollini, S. Schwartz, K. Chan, D. A. Herms, and P. Bonello. 2007. Comparative phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). *J. Chem. Ecol.* 33: 1430–1448.
- Fahrner, S. J., J. P. Lelito, K. Blaedow, G. E. Heimpel, and B. H. Aukema. 2014. Factors affecting the flight capacity of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a classical biological control agent of *Agrilus planipennis* (Coleoptera: Buprestidae). *Environ. Entomol.* 43: 1603–1612.
- Fahrner, S. J., J. P. Lelito, and B. H. Aukema. 2015. The influence of temperature on the flight capacity of emerald ash borer *Agrilus planipennis* and its parasitoid, *Tetrastichus planipennisi*: implications to biological control. *BioControl.* 60: 437–449.
- Haack, R., E. Jendek, H. Liu, K. Marchant, T. Petrice, T. M. Poland, and H. Ye. 2002. The emerald ash borer: a new exotic pest in North America. *Michigan Entomol. Soc. Newsl.* 47: 1–5.
- Hayakawa, Y., and H. Chino. 1981. Temperature dependent interconversion between trehalose in diapausing pupae of *Philosamia cynthia ricini* and *pryeri*. *Insect Biochem.* 11: 43–47.
- Hayakawa, Y., and H. Chino. 1982. Temperature-dependent activation or inactivation of glycogen phosphorylase and synthase of fat body of the silkworm *Philosamia cynthia*: the possible mechanism of the temperature-dependent interconversion between glycogen and trehalose. *Insect Biochem.* 12: 361–366.
- Irwin, J. T., and R. E. Lee. 2000. Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *J. Insect Physiol.* 46: 655–661.
- Kees, A. M., A. R. Hefty, R. C. Venette, S. J. Seybold, and B. H. Aukema. 2017. Flight capacity of the walnut twig beetle (Coleoptera: Scolytidae) on a laboratory flight mill. *Environ. Entomol.* 46: 633–641.
- Kovacs, K. F., R. G. Haight, D. G. McCullough, R. J. Mercader, N. W. Siegert, and A. M. Liebhold. 2010. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. *Ecol. Econ.* 69: 569–578.
- Lelito, J. P., I. Fraser, V. C. Mastro, J. H. Tumlinson, K. Böröczky, and T. C. Baker. 2007. Visually mediated “paratrooper copulations” in the mating behavior of *Agrilus planipennis* (Coleoptera: Buprestidae), a highly destructive invasive pest of North American ash trees. *J. Insect Behav.* 20: 537–552.
- Machial, L. A., B. S. Lindgren, R. W. Steenweg, and B. H. Aukema. 2012. Dispersal of Warren root collar weevils (Coleoptera: Curculionidae) in three types of habitat. *Environ. Entomol.* 41: 578–586.
- McKee, F. R., and B. H. Aukema. 2015. Influence of temperature on the reproductive success, brood development and brood fitness of the eastern larch beetle *Dendroctonus simplex* LeConte. *Agric. For. Entomol.* 17: 102–112.
- McKenney, D. W., J. H. Pedlar, D. Yemshanov, D. B. Lyons, K. L. Campbell, and K. Lawrence. 2012. Estimates of the potential cost of emerald ash borer (*Agrilus planipennis* Fairmaire) in Canadian municipalities. *Arboric. Urban For.* 38: 81–91.
- Miles, P. D., C. L. van der Schaaf, C. Barnett, B. J. Butler, S. W. Crocker, D. D. Gormanson, C. M. Kurtz, T. W. Lister, W. H. McWilliams, R. S. Morin, et al. 2016. Minnesota Forests 2013. Resource Bulletin

- NRS-104. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 134 p.
- Mirth, C. K., and L. M. Riddiford. 2007. Size assessment and growth control: How adult size is determined in insects. *BioEssays*. 29: 344–355.
- Nijhout, H. F. 1975. A threshold size for metamorphosis in the tobacco hornworm, *Manduca sexta* (L.). *Biol. Bull.* 149: 214–225.
- Olson, D.M., H. Fadmiro, J.G. Lundgren, and G.E. Heimpel. 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology*. 25: 17–26.
- Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. *J. For.* 104: 118–124.
- Pureswaran, D. S., and T. M. Poland. 2009. Host selection and feeding preference of *Agrilus planipennis* (Coleoptera: Buprestidae) on ash (*Fraxinus* spp.). *Environ. Entomol.* 38: 757–765.
- Rigsby, C. M., D. N. Showalter, D. A. Herms, J. L. Koch, P. Bonello, and D. Cipollini. 2015. Physiological responses of emerald ash borer larvae to feeding on different ash species reveal putative resistance mechanisms and insect counter-adaptations. *J. Insect Physiol.* 78: 47–54.
- Rodriguez-Saona, C., T. M. Poland, J. R. Miller, L. L. Stelinski, G. G. Grant, P. De Groot, L. Buchan, and L. MacDonald. 2006. Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis*, to induced volatiles of Manchurian ash, *Fraxinus mandshurica*. *Chemoecology*. 16: 75–86.
- Rodriguez-Saona, C. R., J. R. Miller, T. M. Poland, T. M. Kuhn, G. W. Otis, T. Turk, and D. L. Ward. 2007. Behaviors of adult *Agrilus planipennis* (Coleoptera: Buprestidae). *Gt. Lakes Entomol.* 40: 1–16.
- Sisterson, M. S., C. M. Wallis, and D. C. Stenger. 2015. Effects of glassy-winged sharpshooter (Hemiptera: Cicadellidae) feeding, size, and lipid content on egg maturation. *J. Econ. Entomol.* 108: 1–11.
- Sobek-Swant, S., J. C. Crosthwaite, D. B. Lyons, and B. J. Sinclair. 2012. Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer. *Biol. Invasions*. 14: 115–125.
- Sydnor, T. D., M. Bumgardner, and S. Subburayalu. 2011. Community ash densities and economic impact potential of emerald ash borer (*Agrilus planipennis*) in four midwestern states. *Arboric. Urban For.* 37: 84–89.
- Taylor, R.A.J., L.S. Bauer, T.M. Poland, and K.N. Windell. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a flight mill and in free flight. *J. Insect Behavior*. 23: 128–148.
- Van Handel, E. 1985. Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control Assoc.* 1: 302–304.
- Venette, R. C., and M. Abrahamson. 2010. Cold hardiness of emerald ash borer, *Agrilus planipennis*: a new perspective, p. 5. *In* Black Ash Symposium Proceeding Meet. United States Department of Agriculture, Forest Service, Bemidji, MN.
- Wang, X.-Y., Z.-Q. Yang, J. R. Gould, Y.-N. Zhang, G.-J. Liu, and E. Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. *J. Insect Sci.* 10: 1–10.
- Wei, X., W. Yun, R. Reardon, T. H. Sun, L. Min, and J. H. Sun. 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* Fairmaire) in China. *Insect Sci.* 14: 367–373.
- Zera, A. J., and A. Larsen. 2001. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, *Gryllus firmus*. *J. Insect Physiol.* 47: 1147–1160.
- Ziegler, R. 1991. Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca sexta*. *J. Comp. Physiol. B.* 161: 125–31.