BIOLOGY AND LABORATORY REARING OF *SPATHIUS GALINAE* (HYMENOPTERA: BRACONIDAE), A PARASITOID OF THE INVASIVE EMERALD ASH BORER, *AGRILUS PLANIPENNIS* (COLEOPTERA: BUPRESTIDAE)

by

Timothy Joseph Watt

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ABSTRACT

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a rapidly spreading invasive pest that threatens to functionally extirpate North American ash (*Fraxinus* spp.) trees. A classical biological control program for emerald ash borer was initiated and in 2007 three hymenopteran parasitoids were introduced into several US states. Recently, *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae), a newly discovered gregarious idiobiont ectoparasitoid of emerald ash borer larvae, is currently petitioned for environmental release in the US. To gather basic biological information and aid the development of a mass-rearing protocol for *S. galinae*, the following studies were conducted.

First, the effect of emerald ash borer host stage on *S. galinae* critical fitness parameters was investigated. Results showed that when different age (size) larvae developing naturally in the cambium of evergreen (tropical) ash (*Fraxinus uhdei* [Wenzig] Lingelsh) logs were presented to gravid *S. galinae* females, large larvae were parasitized significantly more frequently than small larvae, and broods with more individuals and fewer males were produced when large larvae were used as hosts. In addition, estimates of fitness of progeny based on anatomical measurements were positively associated with host size. Larvae developing naturally were not parasitized by *S. galinae* if they had already bored into the sapwood of the plant and ceased feeding (becoming J-shaped), although when J-shaped larvae were removed from chambers in the sapwood and re-inserted into shallow grooves in a fresh host plant

they were parasitized by *S. galinae*. No pre-pupae or pupae were parasitized by *S. galinae*.

The second study investigated the effect of ambient temperature (15, 20, 25, 30, 35°C) on the developmental and reproductive biology of *S. galinae*. Results showed that at 15°C *S. galinae* developed to 5^{th} instars (cocoons) but then entered diapause, while at 35°C parasitoid eggs became desiccated and did not hatch. Between 20 and 30°C development time was inversely associated with temperature, with adult emergence occurring ≈38, 32, and 25 d after oviposition for 20, 25, and 30°C, respectively. Sex ratios of progeny were not affected by temperature. When newly emerged female parasitoids were provided emerald ash borer hosts throughout their lifetimes, parasitism occurred at all temperatures except 35°C, although parasitism rates were highest, as was reproductive output (fertility), when parasitoids lived at 25°C. Survival was inversely associated with temperature, with ovipositing females at 15°C living for >60 d, while females at 35°C lived for <10 d. Life table analyses showed *S. galinae* had the highest net reproductive rate (R_0=25.7) and greatest capacity for increase (r_c=0.09) when female parasitoids were maintained at 25°C for their lifetimes.

Finally, the effect of parasitoid and host group structure on *S. galinae* fitness was studied by varying the density of parasitoids and hosts exposed in constant size rearing cages. Results showed that when only one emerald ash borer host was exposed in a tropical ash stick to 1, 2, 4, or 8 female parasitoids, *S. galinae* parasitism was positively associated with increasing numbers (densities) of parasitoids, although

brood sizes, sex ratios, and fitness of progeny were not affected. When a constant 1: 1 parasitoid: host ratio was maintained, but the density of parasitoids and hosts exposed together varied from 1-1, 5-5, 10-10, and 20-20 parasitoids-hosts, *S. galinae* parasitism was significantly higher when at least 10 female parasitoids were exposed to at least 10 late instar emerald ash borer larvae. Furthermore, the number of progeny produced per female parasitoid was greater when high parasitoid-host densities were used.

Chapter 1

GENERAL INTRODUCTION

Human-assisted biotic invasions by non-native species threaten many of the world's ecosystems; few habitats on earth remain free of species introduced by humans (Mack et al. 2000, Sala et al. 2000). Although people have served as agents of species dispersal for thousands of years, improvements in technology over the past century have vastly increased the rate at which humans, and their goods and services, move about the globe. Today, global commerce is widely recognized as the primary driver of biological invasions (Westphal et al. 2008). The problems posed by invasive species are far reaching, but range from the loss of aesthetic beauty and economic damage to important crops, to collapses of vital ecosystem functions, disruptions in evolutionary processes, and outright extinctions of species (Mack et al. 2000, Boyd et al. 2013). It is also increasingly recognized that invasive species pose serious threats to human health (Pimentel et al. 2005). In the United States alone, an estimated 50,000 non-native species have been accidentally or intentionally introduced, with a cost of over 130 billion dollars in environmental damages (Pimentel et al. 2000).

Among the most damaging and long-lasting biological invasions are those of insect invaders. Since 1860, non-native insects have been accumulating in North American forests at a rate of 2.5 species per year; today more than 450 nonindigenous

forest insects exist in the United States (Aukema et al. 2010). Recently, a highly invasive forest pest, the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), was accidentally introduced to the United States and Canada. Although its method of introduction is unconfirmed, it is believed that immature emerald ash borers were concealed in wooden shipping crates or pallets of Asian origin (Haack et al. 2002).

Emerald ash borer was originally detected in southeast Michigan in 2002, though dendrochronological evidence indicates it probably established there sometime in the early 1990's (Siegert et al. 2007). Since its detection, emerald ash borer has rapidly dispersed throughout the Midwest and northeastern United States, including 2 Canadian provinces, facilitated primarily by human-induced transport (Muirhead et al. 2006). The spread of emerald ash borer threatens to functionally extirpate North American ash (*Fraxinus* spp.) trees, causing ecological and economic devastation potentially exceeding that of chestnut blight and Dutch elm disease (Kovacs et al. 2010, Herms and McCullough 2014). Emerald ash borer has already killed millions of ash trees throughout the continental US; in southeast Michigan ≥99% of ash trees over 2.5 cm in diameter have been lost (Herms and McCullough 2014).

Ash is an important component of many forest types; at least 16 ash species are distributed throughout a wide variety of habitat types in North America (Harlow et al. 1996, MacFarlane and Meyer 2005). In the US more than 8 billion ash trees are estimated to occur in forests, and several million more have been planted in urban areas as ornamental and street trees (Poland and McCullough 2006). Ash trees provide

food for at least 282 species of arthropods, including 43 known to feed exclusively on ash (Gandhi and Herms 2010). Urban ash trees provide valuable shade for homes and buildings, helping to reduce energy consumption (Nowak 2010). Green and white ash are prized sawtimber species in the eastern US (Cappaert et al. 2005), supplying raw material for furniture, hardwood flooring, and even Major League Baseball bats (Drane et al 2012). In 2006, the total value of all ash in the US was estimated to be nearly one quarter of one trillion dollars (Poland and McCullough 2006).

The larval stage of the emerald ash borer life cycle is the most devastating to ash trees. Larvae usually overwinter as fourth instar, non-feeding J-shaped larvae, termed "J-larvae" by Duan et al. (2010), or prepupae. In the late spring, pupation begins and adults emerge from D-shaped exit holes in the early summer, feeding on the leaves of nearby ash trees and causing only minimal defoliation. After 1-2 wk, eggs are deposited in bark crevices and within two weeks neonate larvae hatch out. Larvae then develop through four instars by feeding on the cambium layer of the tree, creating serpentine galleries that essentially girdle the tree by cutting off its primary mode of nutrient transport. In high densities, emerald ash borer larvae can overwhelm large, mature ash trees in as little as 2-4 years (Scarr et al. 2010).

Early attempts to combat emerald ash borer focused on local eradication and the implementation of state and county quarantines that regulated movements of wood products (e.g. firewood), although these efforts proved mostly fruitless. The application of systemic insecticides, while effective when applied to trees prior to heavy emerald ash borer infestations, are expensive and impractical for forest settings.

Alternatively, classical biological control—the use of foreign natural enemies to reduce target pest densities—was adopted by the US Department of Agriculture as a strategy for mitigating damages caused by emerald ash borer. Though concerns about classical biological control have been voiced (see Simberloff and Stiling 1996, Simberloff 2012), this method has shown the ability to provide safe and relatively cost-effective pest suppression, especially in natural systems like forests (Parry 2009, Van Driesche et al. 2010). In some situations, the benefits of biological control may far outnumber the costs (Bale et al. 2008).

To date, classical biological control of emerald ash borer in the US has focused on the introduction of three hymenopteran parasitoids: the egg parasitoid *Oobius agrili* Zhang and Huang (Encrytidae); and two larval parasitoids, *Tetrastichus planipennisi* Yang (Eulophidae) and *Spathius agrili* Yang (Braconidae). In 2007 these parasitoids were approved for release, and large liberations continue in several midwestern and northeastern states (Lelito et al. 2013). Recently, *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae), a newly discovered gregarious idiobiont ectoparasitoid of late instar emerald ash borer larvae, is petitioned for environmental release in the US (Gould and Duan 2013). *S. galinae* is native to the Russian Far East, and may be a good candidate for biological control in the Northeast and Midwest US where ash is most abundant (Duan et al. 2012).

A key element of effective biological control programs using insect natural enemies is the ability to produce high-quality organisms in a laboratory or insectary setting. When rearing insect natural enemies the goal is usually to produce maximum

numbers of high quality agents while minimizing costs associated with their production (Singh 1982, Waage et al. 1985). This is necessary to provide large numbers of natural enemies to be used for basic biological studies, as well as for environmental release (Singh 1982). Studying the biology of the natural enemy, in turn, can provide useful information that may be used to optimize mass-rearing efforts. Additionally, information obtained from biological studies is used to improve natural enemy release practices and aid field investigations.

To better understand the biology of *S. galinae*, and to facilitate its mass-production, the following laboratory studies were conducted. First, the effect of immature emerald ash borer stages on *S. galinae* critical fitness parameters were investigated. The next study investigated the effect of ambient temperature (15, 20, 25, 30, and 35°C) on the reproductive and developmental biology of *S. galinae*. Finally, the effect of parasitoid and host group structure on *S. galinae* fitness was studied. The results will be used to develop a mass-rearing protocol for *S. galinae*, and should be useful for guiding decisions pertaining to the release of this parasitoid to provide biological control of emerald ash borer in natural settings.

Chapter 2

INFLUENCE OF HOST AGE ON CRITICAL FITNESS PARAMETERS OF *SPATHIUS GALINAE*

Introduction

Many insect parasitoids specialize on a particular developmental stage (egg, larva, or pupa, rarely adult) of their host's life cycle. Furthermore, there may be an optimal size or age within the host stage for maximizing oviposition outcomes (parasitoid fitness) such as the number of progeny produced and the sex ratio of eggs laid (Chabora and Pimentel 1966, Iwasa et al. 1984, Godfray 1994). Identifying the optimal host stage, size, or age for maximizing parasitoid fitness is particularly important when attempting to develop rearing protocols for the purposes of biological control. Indeed, the success of many biological control programs depends largely on minimizing costs associated with rearing the natural enemy (Heinz 1998, Ode and Heinz 2002). For example, one largely prohibitive cost of rearing hymenopteran parasitoids is the overproduction of males (Heinz 1998), which can result from poor host quality (e.g. too small of hosts), among other factors. Similarly, providing parasitoids with suboptimal host resources may result in diminished host utilization rates or reduced progeny fitness, thereby reducing rearing efficiency and artificially inflating production costs (Cancino and Montoya 2006, Irvin and Hoddle 2006, Lopez

et al. 2009). Identifying the proper host condition for rearing natural enemies is therefore a key element of effective biological control.

The pattern of how host size or stage influences the fitness of parasitoids is well documented (Waage and Ng 1984, Godfray 1994). When a female parasitoid attacks a host, she must decide how many eggs to lay, as well as the sex of each egg. Many hymenopteran parasitoids have a haplodiploid sex determination system where females develop from fertilized eggs and males from unfertilized eggs. For solitary parasitoids, theory suggests that female eggs should be allocated to large hosts and male eggs to small hosts due to relative gains in fitness among male and female offspring (Charnov et al. 1981, Charnov 1982). For gregarious parasitoids that produce multiple individuals in a brood, the number of eggs laid per host (clutch size), and the corresponding sex ratio, may also vary with host size (King 1987, Gordh et al. 1999, Bezemer and Mills 2003).

Spathius galinae Belokobylskij and Strazenac (Hymenoptera: Braconidae) is a gregarious idiobiont ectoparasitoid of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Currently, methods are being developed to rear this parasitoid for classical biological control of the invasive emerald ash borer in the United States (Gould and Duan 2013). In its native range (Russian Far East), *S. galinae* attacks primarily third and fourth (occasionally second) instar emerald ash borer larvae, with 8 — 12 (range 1 — 16) progeny resulting from a single host (Belokobylskij et al. 2012), and is an important natural enemy of emerald ash borer on

the introduced North American green ash, *Fraxinus pennsylvanica* Marshall, in Russia (Duan et al. 2012).

In North America emerald ash borer is a highly invasive pest. Since its accidental introduction into southeast Michigan in the mid-1990's (Cappaert et al. 2005), emerald ash borer has rapidly spread to 21 other US states and 2 Canadian provinces (Emerald Ash Borer Info 2014), devastating both forest and urban ash (*Fraxinus* spp.) tree populations, and causing billions of dollars in economic damage (Kovacs et al. 2010, see review in Herms and McCullough 2014). Classical biological control for emerald ash borer has centered on the establishment of three hymenopteran parasitoids: the egg parasitoid *Oobius agrili* Zhang and Huang (Encrytidae), the larval endoparasitoid *Tetrastichus planipennisi* Yang (Eulophidae), and the larval ectoparasitoid *Spathius agrili* Yang (Braconidae). To complement these efforts, environmental releases of *S. galinae* in the US are anticipated for 2015 (Gould and Duan 2013).

The emerald ash borer life cycle is complex and involves several distinct stages (see Wang et al. 2010b for a complete description). After eclosion from the egg, neonate larvae develop through four instars. Although the morphology of each larval instar is similar (Chamorro et al. 2012), late (3^{rd} and 4^{th}) instar larvae have significantly more biomass than the early (1^{st} and 2^{nd}) instar larvae (Duan et al. 2013a., Wang et al. 2013). The fourth stadium terminates in a non-feeding phase where the larva bores into the sapwood (or thick outer bark) and excavates its pupal chamber in which it folds into a J-shape for obligatory diapause (overwintering). Larvae in this

stage are termed J-larvae, and may be differentiated from fourth instar larvae as well as prepupae (Duan et al. 2010). After the period of obligatory diapause, J-larvae begin pupation, first by straightening and compressing in appearance, becoming prepupae, and then developing red eye spots and imaginal disks that indicate the pupal stage.

To date, no studies have examined the possible influence different emerald ash borer life stages may have on the fitness of *S. galinae.* The objective of this study therefore was to determine how emerald ash borer larval age (size) and stage affect host utilization and critical fitness parameters of *S. galinae,* with the aim of aiding in the development of effective laboratory rearing protocols. The results from this study will be important for determining the optimal emerald ash borer size and stage for rearing *S. galinae,* and may be useful when considering field releases of this parasitoid.

Materials and Methods

Preparation of emerald ash borer host stages

Two methods were used for obtaining the desired stages of immature emerald ash borer hosts for exposure to *S. galinae*. To obtain larvae, emerald ash borer eggs (≤10 d old at 25 ± 1°C) were used to infest freshly cut, greenhouse cultivated tropical ash logs according to methods described in Duan et al. (2011, 2013a). Briefly, 12 emerald ash borer eggs were placed onto singular tropical ash logs (25 cm length x 2-3 cm diameter), targeting seven to ten viable host larvae per log. Tropical ash logs (infested with emerald ash borer eggs) were then placed on distilled water-saturated floral foam bricks (OASIS, Smithers-Oasis Company, Kent, OH), with a 0.1%

methylparaben solution to prevent mold, and housed inside ventilated clear plastic bins (58 x 41 x 31 cm, Sterilite Co., Townsend, MA). Logs were incubated in an AR-360 environmental growth chamber (Percival Scientific, Perry, IA) at 25 ± 1°C (≈65% RH, 16L: 8D photoperiod) until the target age for parasitoid exposure. When the logs (containing host larvae) reached the target age (3.5, 5, 7, and 10 wk) they were removed from incubation and prepared for parasitoid exposure.

Late stage emerald ash borer hosts (J-larvae, prepupae, and pupae) were obtained from field-collected green ash trees felled in southern Maryland. Several ≈1m long sections of green ash trees were cut and collected in late fall (late October through late November) and spring (early to mid-April) and transported to a Maryland Dept. of Agriculture warehouse in Cheltenham, MD. Log sections collected in fall usually contained J-larvae and were stored at ≈3°C in a large walk-in cooler (Polar King International, Ft. Wayne, IN) to maintain this stage. Logs collected in the spring (which had been previously exposed to winter conditions) usually contained prepupae and pupae. To dissect out the emerald ash borer J-larvae, prepupae, and pupae, a drawknife (to peel back the outer bark) and chisel and hammer were used to expose the insects in chambers in the sapwood or outer bark. The insects were removed using soft forceps and then placed in petri dishes (50x9mm with tight-fit lid; Falcon®, Becton Dickinson, Franklin Lakes, NJ) for transportation to the Beneficial Insects Introduction Rearing Laboratory (BIIRL) in Newark, DE. Once in the laboratory, individual insects were examined for the presence of damage, mold, or parasitism, and

clean (healthy) insects were identified by stage (J-larvae, prepupae, or pupae) prior to parasitoid exposure.

Parasitoid colony

All *S. galinae* used throughout the study were $F_{8\text{-}10}$ progeny from a founder colony collected near Vladivostok, Russia (Russian Far East) in the fall of 2010 (Duan et al. 2012). Before use in exposure assays, multiple (≈20-40) male and female parasitoids were held in ventilated acrylic containers (20 cm height x 12 cm diameter) at a ≈1: 2 male: female ratio and maintained in environmental chambers at 25 ± 1°C (≈65% RH, 16: 8 h L: D photoperiod) for mating. Mesh screens on the containers were routinely streaked with clover honey (food source) and a small 10-dram plastic vial (US plastics, Lima, OH) was fitted with a water-saturated dental wick (Richmond Dental, Charlotte, NC) to provide an accessible water source. All parasitoids were 2-3 weeks old, gravid, and naïve (i.e. no previous exposure to host larvae).

Parasitoid exposure

All exposures were conducted in environmental chambers set at the same conditions as for colony maintenance. Emerald ash borer larval hosts were exposed to *S. galinae* in the following manner. Tropical ash logs were placed individually in a 120 cc specimen cup (Dynarex Co., Orangeburg, NY) with water-saturated rock wool (Grodan™, Netherlands) to provide a water source. The base (bottom 2 cm) of the log was wrapped to the cup with Parafilm (BEMIS, BEMIS flexible packaging, Neenah, WI) to stabilize the log. The logs were then placed in the exposure arenas, which consisted of a 20 x 10 cm ventilated cylindrical cage tightly fastened to the inside lip

of a 1 L white polypropylene cup (Berry Plastics Co., Evansville, IN). Each exposure arena contained one log. Ten female and 2-3 male parasitoids (maintaining ≈1:1 parasitoid: host ratio) were randomly transferred to an exposure arena using a fine camel paintbrush (Simply Simmons 25 Round, Daler-Rowney, Cranbury, NJ). The logs containing larval hosts were exposed to parasitoids for 7 days at 25°C (≈65% RH, 16: 8 h L: D photoperiod). After this period, parasitoid-exposed logs were removed from exposure arenas and placed in the environmental chambers for progeny emergence.

Late stage emerald ash borer (J-larvae, prepupae, and pupae) hosts were inserted into a ≈7 mm (diameter) x ≈4 mm (depth) groove cut into the surface of a 9 cm (length) x 3 cm (diameter) green ash stick using the methods described in Ulyshen et al. (2010a). A single log holding either four or five J-larvae, prepupae, or pupae was placed in an 18.5 x 13 x 10 cm polystyrene crisper box (Tri-State Plastics, Dixon, KY) exposure arena. Four to five female and 1-2 male parasitoids were then randomly assigned to each exposure arena maintaining ≈1:1 parasitoid: host ratio as before. Hosts were exposed to parasitoids for 7 d, after which parasitoids were removed and logs were placed in environmental chambers for progeny emergence.

Relationship between emerald ash borer age, size, and instars

Due to emerald ash borer's cryptic larval habitat (concealed beneath the bark), it was not possible to directly measure the size of emerald ash borer larvae in logs prior to parasitoid exposure. Consequently, the age of the emerald ash borer larvae was used as a proximate cue for host size in this study. Previous studies have found a

positive relationship between emerald ash borer larval weight and gallery width (Wang et al. 2008), as well as between emerald ash borer weight, gallery width, and instars (Duan et al. 2013a). To determine the relationship between emerald ash borer larval age, size, and instar in this study, larval subsamples were dissected from control logs that were prepared simultaneously with the logs prepared for parasitoid exposure. Forty larvae (10 for each host age) were randomly removed from these logs and weighed using an electronic balance (AB135-S/FACT, Mettler Toledo AG Laboratory Weighing and Technology, Greifensee, Switzerland) and measured for gallery width at the widest position of the head using an electronic caliper (No. 147-Digital Fractional caliper Stainless Steel UltraTech, General Tools and Instruments, NewYork, NY). These measurements were then used to infer the instar of each larva.

Effect of host larval age on critical fitness parameters of *S. galinae*

This experiment was designed to test the effect of different emerald ash borer host larval sizes on the host utilization and fitness of *S. galinae*. As stated above, it was not possible to know the exact size of larvae in tropical ash logs prior to exposure to parasitoids, so age was used as a proximate variable for size. We tested four larval age classes: 3.5, 5, 7, and 10 wk-old. The age of larvae in each log was known based on the number of days elapsed since the original oviposition of the emerald ash borer eggs used to infest the logs. Each host age class (treatment) was replicated 22 times using a "no choice" random block design. Each replicate (block) consisted of all age treatments of the host larvae reared in tropical ash logs and exposed to the same batch (i.e. reared under similar conditions and of similar age) of adult parasitoids at the same

time. Two weeks after the last progeny emergence, the parasitoid-exposed logs were dissected using a utility knife to remove the outer bark and expose the host galleries. The galleries of parasitized hosts were easily observed because of the presence of silken cocoons spun by the mature parasitoid larvae prior to pupation. We determined the instar of each parasitized host larva based on the width of the gallery where parasitism was observed. As an idiobiont *S. galinae* paralyzes its host during oviposition, which immediately ceases host development. Wang et al. (2013) found that emerald ash borer gallery width may be used to estimate the size and instar of parasitized emerald ash borer larval hosts even after they are consumed by parasitoid progeny.

Upon *S. galinae* self-emergence, all individuals were counted and sexed. One female and one male parasitoid were randomly selected from the first-emerged progeny of every log and placed in a vial of 95% ethyl alcohol for later measurement of anatomical fitness markers (length of body, left hind tibia, and female ovipositor). Although tracking the longevity and fecundity of progeny is often the best method for measuring fitness, several studies have shown that the size of the adult parasitoid may be used as an index of lifetime fitness (Jervis et al. 2005). Fitness measurements were conducted using a stereomicroscope and ocular stage micrometer (50 x 2 microns, PYSER-SGI, Edenbridge, Kent, UK) to calibrate the measurements. For every individual, we first measured the body length from the anterior tip of the head to the posterior tip of the abdomen and then removed the left hind leg at the coxa and measured the length of the tibia. The ovipositor of female specimens was then

separated from the abdomen and length was measured from where the oviduct attaches to the anterior tip of the ovipositor to the posterior end of the ovipositor.

Host utilization of late stage emerald ash borer hosts by *S. galinae*

To test the possibility that late stage emerald ash borer (J-larvae, prepupae and pupae) hosts may be used to rear *S. galinae,* a separate experiment was conducted which involved removing hosts as J-larvae, prepupae, or pupae from field-collected green ash logs and re-inserting them into a new green ash substrate (9 x 3 cm sticks). Each of the treatments (J-larvae, prepupae, and pupae) was replicated twelve times. Parasitoid progeny were allowed to emerge, and any emerged progeny were counted and sexed.

Data analysis

A one-way analysis of variance (ANOVA) was used to determine the relationship between emerald ash borer age and weight, followed by Tukey-Kramer's HSD test to detect significant differences among each host age. The effect of host age and stage on critical fitness parameters (host attack rate, brood size, progeny size, sex ratio, and anatomical measurements of progeny fitness) of *S. galinae* were calculated for each replicate assay as follows. Host attack rate (% parasitism) was calculated by dividing the number of hosts parasitized by the total number of hosts available in the log at the time of parasitoid exposure. Brood size was calculated by dividing the sum of the progeny (male and female) by the total number of parasitized hosts, and sex ratio is reported as the ratio of female to male offspring in the emerged progeny. The anatomical measurements of progeny fitness (length of body, left hind tibia, and

female ovipositor) were measured for each of 22 female and male *S. galinae* as described previously. Because no emerald ash borer prepupae or pupae were parasitized by *S. galinae,* prepupae and pupae were excluded from the analysis. The means of the parameters for all trials were first checked for the assumptions of normality and homogeneity of variance for parametric tests. An arcsine transformation was performed for host attack rate (binomial factor) and sex ratio, whereas brood size was log-transformed. The measurements of progeny fitness met the assumptions and were not transformed. One-way ANOVAs were performed followed by Tukey-Kramer's HSD test for multiple means comparisons among different host age treatments. All analyses were carried out using JMP 10.0.1 (SAS Institute 2012).

Results

Relationship between emerald ash borer age, size, and instar

The mean biomass of emerald ash borer larvae dissected from the control logs was smallest for 3.5-wk host larvae (0. 2 ± 0.4 mg), and largest for 10-wk hosts (109 ± 0.4 mg) (Figure 1A). Larvae were all first or second instars in 3.5-wk old logs, 80% third instars in 5-wk logs, 90% fourth instars in 7-wk logs, and 80% J-larvae in 10-wk old logs (Figure 1B).

Effect of host larval age on critical fitness parameters of *S. galinae*

Between 113 and 144 emerald ash borer host larvae were provided to *S. galinae* in each of the four host age treatments. When larval hosts were exposed to *S. galinae*, 5 and 7-wk hosts were attacked significantly more frequently than 3.5-wk and 10-wk hosts (Table 1). Among only parasitized hosts, 3.5 and 5-wk hosts had only 3 –

4 offspring per host, while 7 and 10-wk hosts had 6 – 7 (Figure 2). Sex ratio was significantly more female biased in logs with 7 and 10-wk hosts than in logs with 3.5 or 5-wk hosts (Figure 3). The anatomical estimates of progeny fitness (measured as length of body, left hind tibia, and female ovipositor) were larger in the 7 and 10-wk hosts than in younger hosts, for both males and females (Figure 4).

Host utilization of emerald ash borer J-larvae, prepupae, and pupae by *S. galinae*

When exposed to J-larvae, prepupae, or pupae inserted into green ash logs, *S. galinae* attacked more than half of the J-larvae, but no prepupae or pupae. Each parasitized J-larva produced a mean of 5.4 parasitoid progeny with a strongly female-biased sex ratio (Table 2).

Discussion

The results of this study demonstrate a clear influence of emerald ash borer larval age (size) and stage on the host utilization and fitness of *S. galinae.* When exposed to emerald ash borer larvae of different ages reared in tropical ash logs, *S. galinae* parasitized 5 and 7-wk (3^{rd} and 4^{th} instar) hosts significantly more often than 3.5-wk (2^{nd} instar) hosts or 10-wk hosts, which were mostly mature J-larvae. Seven and 10-wk hosts produced significantly larger broods (per parasitized host) than 3.5-wk and 5-wk hosts, and sex ratios were significantly more female biased in the 7 and 10-wk hosts. Furthermore, the size of the adult parasitoids emerging from tropical ash logs was greater in the 7 and 10-wk hosts, regardless of sex. For parasitoids, the size of the adult is largely determined by the amount of food consumed as a larva (Godfray 1994). Because *S. galinae* is an idiobiont, the size of the emerald ash borer host at the

time of oviposition represents the maximum available resource for the developing larvae. Thus, adult *S. galinae* size is directly influenced by the size of its emerald ash borer host larva. Although adult fitness is usually best measured directly in terms of lifetime fecundity, many studies have shown significant correlations between female body size, or other anatomical markers such as tibia length, and adult fitness (Waage and Ng 1984, Godfray 1994, Jervis et al. 2005). These studies have found that longevity and fecundity may increase with parasitoid size; large females often carry greater egg loads (Opp and Luck 1986, Rosenheim and Rosen 1991) and may be more effective at locating hosts (Bezemer and Mills 2003) than small females. When rearing *S. galinae*, large females are preferred because: 1) they will have more energy reserves for egg development (synovigeny); 2) they have long ovipositors capable of attacking hosts concealed under the thick bark of mature ash trees; and, 3) they may live longer to parasitize more hosts than small females.

When emerald ash borer larval hosts were reared in tropical ash logs, we found that *S. galinae* parasitized 12% of 10-wk and 23% of 3.5-wk hosts. However of the 10-wk hosts parasitized, broods were largely female-biased and similar in the number of offspring as those produced from 7-wk hosts, which were parasitized 76% of the time, while *S. galinae* broods from 3.5-wk hosts were much more male-biased and had much fewer individuals than those from 7-wk hosts. The reason 10-wk hosts were infrequently parasitized yet produced strongly female-biased broods is because the majority of larvae in these logs were J-larvae. All parasitized 10-wk hosts were fourth instar larvae; no J-larvae were parasitized when reared in tropical ash logs. Ulyshen et

al. (2010b) explained that J-larvae are not naturally attacked by emerald ash borer larval parasitoids because J-larvae have bored into the sapwood of the plant and likely are not detected by foraging parasitoids as J-larvae do not produce the chewing vibrations of actively feeding larvae. This raises the possibility that J-larvae may be useful for rearing *S. galinae* if they could be detected by foraging parasitoids. Indeed, when J-larvae were removed from their chambers in the sapwood of field-collected green ash logs and re-inserted into a new host plant substrate, these larvae again tried to chew a new chamber and *S. galinae* were able to parasitize them more than half of the time, resulting in brood sizes and sex ratios similar to those produced using fourth instar emerald ash borer hosts.

Rearing emerald ash borer larval hosts in the laboratory for the purposes of parasitoid production has proven to be extremely difficult in the years since emerald ash borer biological control programs were initiated. Indeed, only recently have researchers established methods that allow the year-round production of larval hosts (Duan et al. 2011, 2013a). Intimate knowledge of the biology of both the emerald ash borer and its parasitoids is thus crucial to maintaining low production costs such that biological control remains affordable. Here, we show that large, 4th instar emerald ash borer larvae should be the target when rearing *S. galinae.* Within the tropical ash-based emerald ash borer rearing system (Duan et al. 2011), however, there is variation in development times. Under standard rearing conditions (25°C, ≈65% RH, 16:8 L:D), 50% of emerald ash borer larvae normally molt to the fourth stadium in ≈6wks (Duan et al. 2013a), but timing may vary by several days depending on the health and vigor

of the host plant, or host larval density (Duan et al. 2013c). In mass-rearing situations, where larval hosts are often produced in surplus, excess fourth instar larvae may turn to J-larvae prior to use for parasitoid production. The finding that J-larvae may be removed from their host plants and re-inserted into a new host plant substrate, and can then successfully be used to rear *S. galinae* is critically important for reducing the cost of its production.

Belokobylskij et al. (2012) reported a native brood size of 8 — 12 for *S. galinae.* In our study, the largest broods observed were between 6 — 7 individuals. A number of studies suggest that observations on the number of eggs laid per host (i.e. clutch size) may be lower in the laboratory, where conditions are optimal and hosts are not perceived to be limiting, than in the field, where life expectancy is shorter and host location requires significantly more energy (Rosenheim and Rosen 1991, Godfray 1994, Bezemer and Mills 2003). Alternatively, the lower brood size we observed in this study could be the result of using only naïve females. Charnov (1982) explains that host size is a relative rather than an absolute parameter, and oviposition decisions made by a parent female may largely be based on prior experience. Thus, parameters of host utilization, such as the number and sex ratio of eggs laid, may be optimized by parasitoids that have experienced successful parasitism events. By using groups of female parasitoids in several consecutive exposure periods, it may be possible to optimize parameters such as brood size, sex ratio, and progeny fitness. Heinz (1998) was able to decrease the proportion of male progeny in the mass-reared pteromalid wasp *Catolaccus grandis* (Burks) by providing the same group of wasps sequentially

larger hosts over consecutive exposure days. Similarly, Ode and Heinz (2002) improved female offspring sex ratio of the mass-reared agromyzid leafminer parasitoid *Diglyphus isaea* (Walker) by providing female parasitoids with sequentially larger hosts. By providing groups of *S. galinae* females with sequentially larger emerald ash borer hosts over their lifetimes, it may be possible to increase the proportion of female offspring produced. This would be an important finding because such a method could be incorporated into rearing protocols and could help increase rearing efficiency (reducing costs) by reducing the number of males produced.

There are several reasons for considering the introduction of *S. galinae* to the US. First, the origin of collection for *S. galinae* (Vladivostok, Russia) has a high climate matching index (≥0.75) with northeast and north central US where emerald ash borer densities are currently highest (Duan et al. 2012). Compared with *S. agrili*, which originates ≈800 km south near Tianjian, China, and has a climate matching index of 0.60 for these regions (USDA APHIS 2007), *S. galinae* may represent a more suitable candidate for introduction where *S. agrili* has had little success establishing. Secondly, whereas *T. planipennisi* has an ovipositor length of 2 — 2.5 mm (Duan and Oppel 2012) and is capable of attacking larvae concealed in ash trees with ≤11.2 cm dbh (Abell et al. 2012), *S. galinae* has an ovipositor length of 4 — 5 mm and is known to attack emerald ash borer larvae in much larger ash trees with dbh ranging from 14 cm to ≥36 cm (Duan et al. 2012, Belokobylskij et al. 2012). *Atanycolus* spp., a native group of braconid found extensively attacking emerald ash borer larvae in Michigan, have ovipositors 6 — 8 mm in length and are able to attack hosts in trees of ≥57.4 cm

dbh (Abell et al. 2012), however these parasitoids are often found attacking only high density emerald ash borer populations in areas where ash tree mortality is also high. *S. galinae* may therefore be an important biological control agent for protecting ash trees of reproductive age that are not infested with high densities of emerald ash borer. Recent laboratory studies by Yang et al. (2012) also showed that *S. galinae* has a strong ability to discriminate between healthy emerald ash borer larvae and larvae parasitized by *T. planipennisi,* and the authors concluded that the presence of *S. galinae* in the field would likely have limited effects on the competitive interactions between these two species. Further possible interactions between *S. galinae* and other introduced and native parasitoids should be investigated.

The finding that *S. galinae* prefers large, 4^{th} instar hosts, and does not naturally attack emerald ash borer in the J-larva, pre-pupa, or pupa stage, has clear implications for releasing it in the field. If parasitoids are released early in the season when emerald ash borer larvae are mostly small, parasitism by *S. galinae* may result in fewer female progeny or female progeny with short ovipositors, rendering them incapable of attacking emerald ash borer hosts in large trees. Similarly, if released late in the season, many emerald ash borers will have become J-larvae and remain undetected in ash trees by *S. galinae*, resulting in fewer overwintering individuals available for biological control the following season. Thus, a thorough understanding of the phenology of emerald ash borer is necessary for establishing *S. galinae* where emerald ash borer exists.

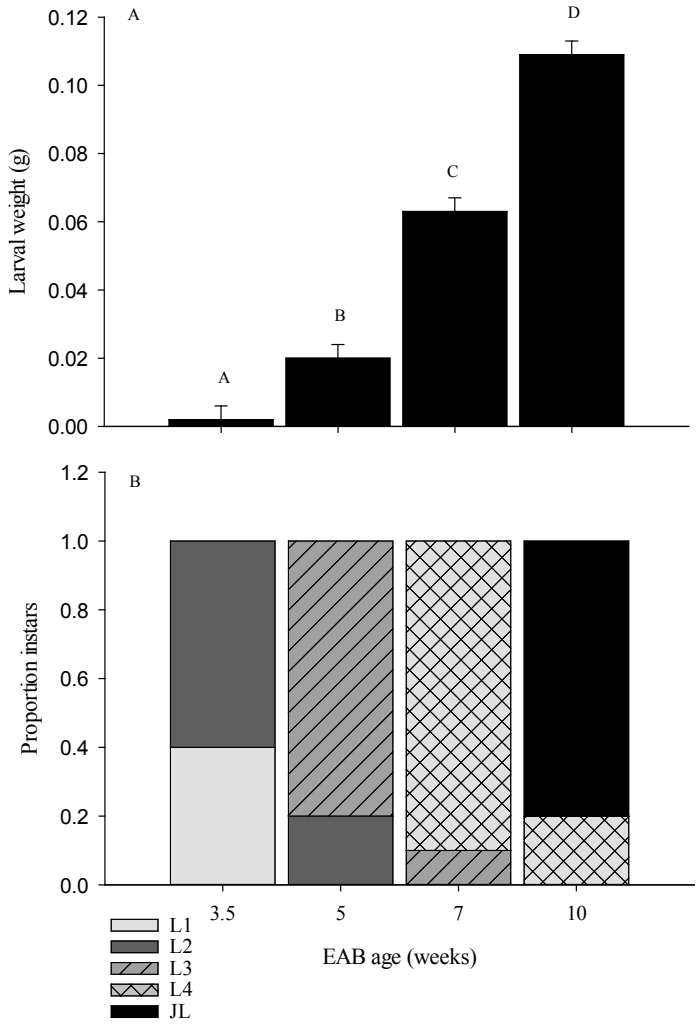

Figure 1. (A) Mean (±SE) biomass of different age emerald ash borer (EAB) larvae. Bars with different letters are significantly different ($F=124.8$, $df=39$, $P<0.0001$; $\alpha=0.05$). (B) Distribution of EAB larval instars by age (L1 – L4, larval instars 1 – 4; JL, J-larvae).

Table 1. *Spathius galinae* parasitism rates on different age emerald ash borer (EAB) larval hosts.

EAB age (no. wk at 25°C)	No. available hosts	No. hosts parasitized	% parasitism (mean ± SE)[a]	Total no. parasitized larvae[b]				
				L1	L2	L3	L4	JL
3.5	113	26	23.2 ± 6.2b	0	23	3	0	0
5	144	103	68.0 ± 5.8a	0	24	79	0	0
7	144	110	75.9 ± 4.7a	0	0	4	106	0
10	133	18	12.3 ± 3.2c	0	0	0	18	0

[a] Values with different letters are significantly different ($F=34.7$, $df=3$, $P<0.0001$) ($\alpha=0.05$).
[b] L1 – L4, larval instars 1 – 4; JL, J-larvae.

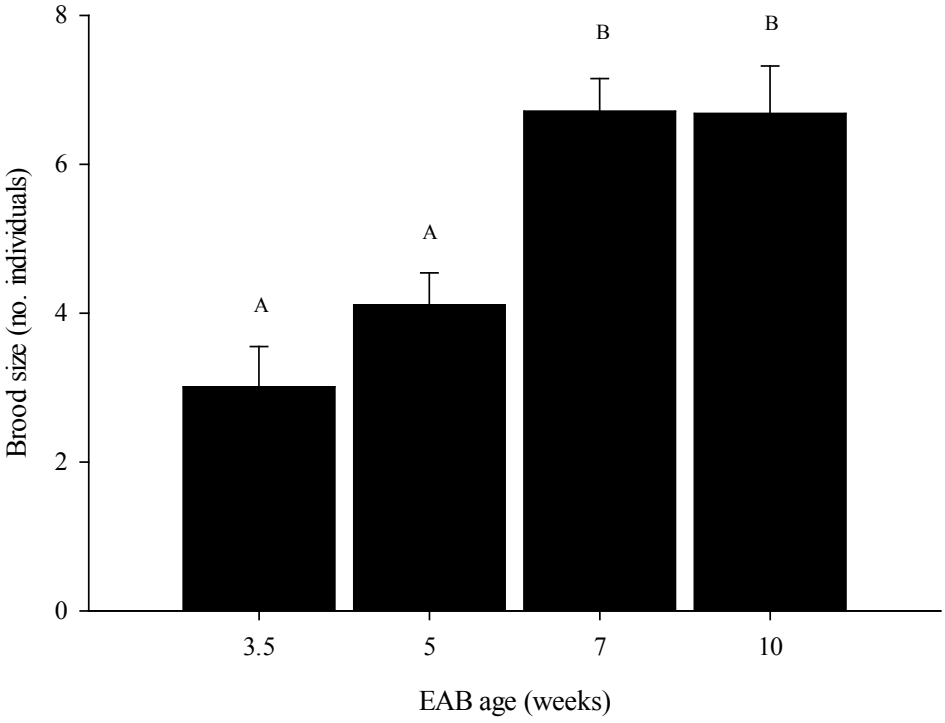

Figure 2. Mean (±SE) brood size (no. offspring per host) of *Spathius galinae* by EAB host age. Bars with different letters are significantly different ($F=13.2$, $df=3$, $P<0.0001$; $\alpha=0.05$).

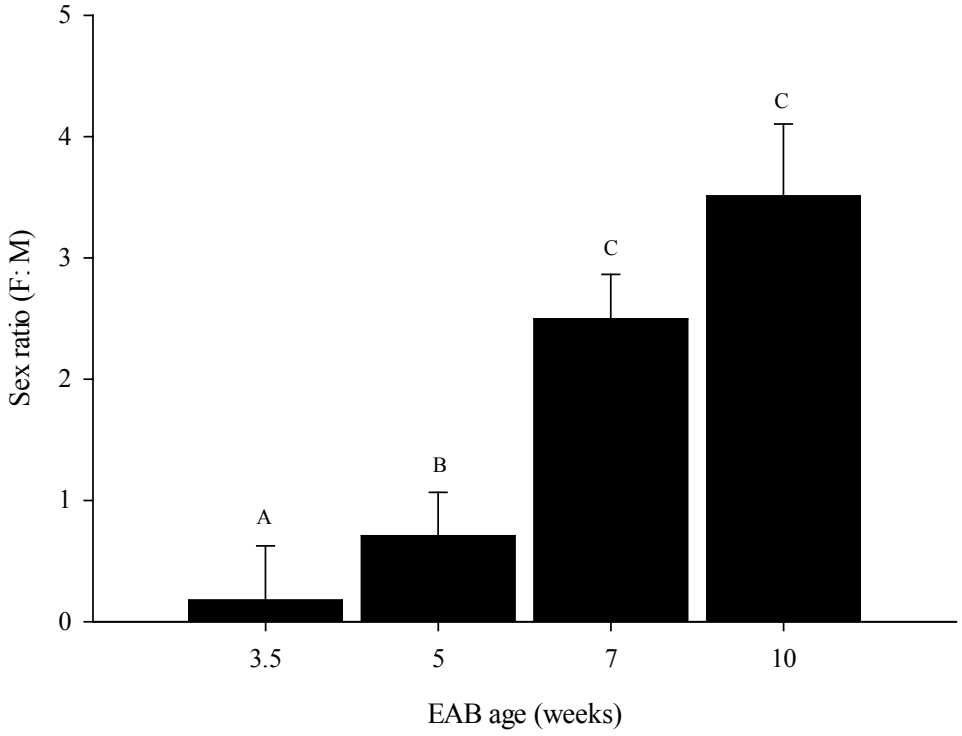

Figure 3. Mean (±SE) sex ratio of *Spathius galinae* progeny by EAB host age. Bars with different letters are significantly different ($F=18.6$; $df=3$, $P<0.0001$; $\alpha=0.05$).

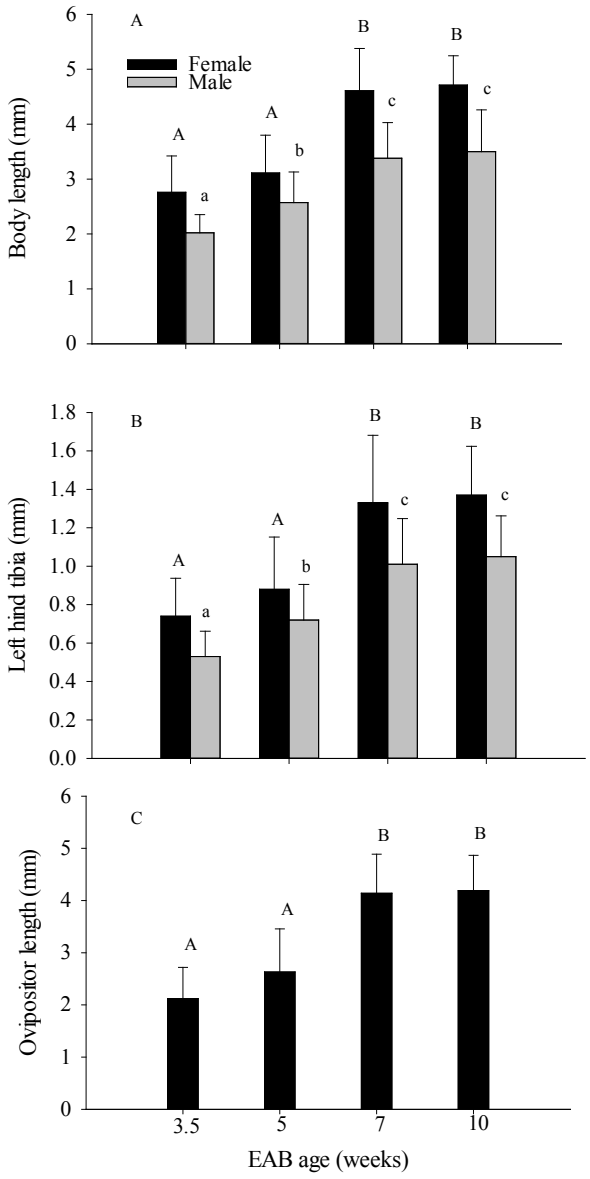

Figure 4. Anatomical measurements of *Spathius galinae* fitness of progeny by EAB host age. (A) Mean (±SE) body length of males ($F=21.0$, $df=3$, $P<0.0001$) and females ($F=22.5$, $df=3$, $P<0.0001$). (B) Mean (±SE) female ($F=11.7$, $df=3$, $P<0.0001$) and male ($F=22.9$, $df=3$, $P<0.0001$) length of left hind tibia. (C) Mean (±SE) female ovipositor length ($F=18.3$, $df=3$, $P<0.0001$). Bars with different letters are significantly different ($\alpha=0.05$).

Table 2. *Spathius galinae* parasitism rates on artificially inserted late stage EAB hosts in green ash logs.

EAB stage	N (available hosts)	% hosts parasitized (mean ± SE)	Brood size (mean ± SE)	Sex ratio (f:m) (mean ± SE)
J-larvae	52	53.33 ± 4.77	5.44 ± 0.35	3.30 ± 0.48
Prepupae	42	0	0	0
Pupae	46	0	0	0

Chapter 3

EFFECT OF AMBIENT TEMPERATURE ON THE REPRODUCTIVE AND DEVELOPMENTAL BIOLOGY OF *SPATHIUS GALINAE*

Introduction

When evaluating the suitability of an insect natural enemy for classical biological control, one important consideration is how climate might influence the performance of the candidate natural enemy in the introduced region (Hoelmer and Kirk 2005). For example, the invasion success of a parasitoid may reasonably be predicted if the impacts of temperature on estimates of population growth are known (Pilkington et al. 2014). Of all the climatic components, temperature exerts the most influence on insect performance (Infante 2000, Bale et al. 2002, Ju et al. 2011, Baffoe et al. 2012), and its effects have been widely studied (Dixon et al. 2009). Understanding how temperature impacts the reproductive and developmental biology of parasitoids is important not only when evaluating establishment potential (Pilkington and Hoddle 2006), but identifying the thermal requirements that optimize development and reproduction is essential for developing effective protocols to mass-rear agents in the laboratory (Qiu et al. 2012).

Insects are ectothermic; their activities and life processes are highly regulated by ambient temperature. Physiological and metabolic functions, including growth and

development of immature stages and adult egg production, are directly influenced by temperature (Patton 1963, Taylor 1981). Most insects have a range of temperatures within which, and in the absence of limiting factors (e.g. host availability or predation), developmental rates and reproduction are optimized. These species-specific optimal temperature ranges are sometimes called 'thermal windows' (Dixon et al. 2009). In addition to the thermal window there are also high and low thermal thresholds beyond which development and reproduction may cease altogether. Typically, the rate of development increases from zero at the low threshold, becoming maximal in the optimal range, followed by a rapid decline until the upper limit is reached (Lactin et al. 1995). Fertility, a measure of reproductive output, also usually peaks in the optimal range, with temperatures above and below the optimal range initiating stress responses that reduce ovigenesis and may greatly limit survival (Jervis et al. 2005).

A commonly used tool for studying the effects of temperature on the reproductive and developmental biology of parasitoids used in biological control is a fertility life table (Force and Messenger 1964, Van den Bosch et al. 1982, Staubli Dreyer et al. 1997, Pratissoli and Parra 2000). Fertility life tables provide information about key demographic parameters, such as the net reproductive rate (R_0), generation time (T_c), innate capacity for increase (r_c), finite rate of increase (λ), and population doubling time (T_d), and show how populations change as a result of experiencing different temperatures. These data assist estimates of when temperatures favor

population growth in the field, and may also be used to define the conditions that maximize outputs of mass-rearing (Qiu et al. 2012, Gomez-Torrez et al. 2012).

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive pest of great ecological and economic importance in North America (Herms and McCullough 2014). Originally established in southeast Michigan in the mid-1990's (Cappaert et al. 2005, Siegert 2007), this wood-boring beetle continues to rapidly expanded its territory. Currently, emerald ash borer is found as far north as Houghton, MI (47.1°N latitude,) and as far south as Fulton County, GA (33.7°N latitude; Emerald Ash Borer Info 2014). Efforts to combat its spread using classical biological control have focused on the introduction of three hymenopteran parasitoids: *Oobius agrili* Zhang and Huang (Encrytidae), *Tetrastichus planipennisi* Yang (Eulophidae), and *Spathius agrili* Yang (Braconidae). While *T. planipennisi* (and possibly *O. agrili,* J. J. Duan and K. J. Abell, personal communication) has successfully established and is spreading in several regions (Duan et al. 2013b), *S. agrili* has rarely been recovered in many of the areas it has been released (Lelito et al. 2013). The origin of collection for *S. agrili* is Tianjian, China (39.1°N latitude, Yang et al. 2010); in North America the majority of emerald ash borer infestations are north of this latitude. Possibly, *S. agrili* may be unable to survive overwintering conditions where it has been introduced; no further releases of *S. agrili* are planned north of 40°N latitude (Lelito et al. 2013).

Recently, a congener of *S. agrili*, *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae), has been petitioned for release in the US (Gould and Duan 2013). *S. galinae* was collected near Vladivostok, Russia (43.1°N latitude), and, unlike *S. agrili*, has a high climate matching index for the north-central and northeast US where ash (*Fraxinus* spp.) are most abundant (Duan et al. 2012). *S. galinae* is a gregarious idiobiont ectoparasitoid of late instar emerald ash borer larvae. Duan et al. (2014) investigated the developmental and reproductive biology of *S. galinae* in the laboratory under standard rearing conditions (25 ± 1°C), however no studies have specifically addressed the role of temperature in regulating this parasitoids' reproductive and developmental parameters. Therefore, this study sought to investigate how temperature impacts the reproductive and developmental biology of *S. galinae,* and to use these data to construct fertility life tables on key population demographic parameters. The information derived from this study will be necessary for developing an effective mass-rearing protocol, and may assist predictions about the establishment of *S. galinae* in North America, especially in response to changing climatic conditions.

Materials and Methods

Emerald ash borer larvae

Emerald ash borer larvae were reared at constant 25°C in small tropical (evergreen) ash (*Fraxinus uhdei* [Wenzig] Lingelsh) sticks or large logs according to methods described in Duan et al. (2011, 2013a). For small ash sticks (9 cm length x 1-1.5 cm diameter), a single fertilized emerald ash borer egg was placed against the

surface of a tropical ash stick and tautly wrapped with Parafilm (BEMIS, BEMIS flexible packaging, Neenah, WI) to secure it to the stick until hatching. For large logs (10-12 cm length x 3-4 cm diameter), five fertilized emerald ash borer eggs (targeting 3 — 4 larvae per log) were similarly wrapped to a log. Larvae developed naturally within the sticks or logs inside of AR-360 environmental growth chambers (Percival Scientific, Perry, IA), as described in Chapter 1. Duan et al. (2013a) showed that emerald ash borer larvae should reach late (3^{rd} – 4^{th}) instars ≈7 weeks after oviposition when reared at 25°C. After 7 wk, sticks and logs were checked for evidence of host larvae by shaving back a thin strip of bark to expose the frass from the larval gallery. Sticks and logs containing larvae were wrapped at the base with wet paper towel (sticks without larvae were discarded), stood on small petri dishes (Falcon®, Becton Dickinson, Franklin Lakes, NJ) and then wrapped at the base with Parafilm and prepared for experimental use.

Temperature-dependent development of *S. galinae*

Groups (15-20) of sticks (each with a single host larva) were exposed to naïve, gravid, ≈2 wk-old female parasitoids for 24 h at a parasitoid: host ratio of 6: 1 to ensure parasitism of hosts. An 18.5 x 13 x 10 cm polystyrene crisper box (Tri-State Plastics, Dixon, KY) was used as the exposure arena. After the exposure period, sticks (containing parasitized hosts) were removed from the exposure arena and randomly assigned to emergence containers (ventilated, clear plastic 1 L SOLO cups with mesh-screened lids; SOLO Cup Co., Urbana, IL), which were previously assigned to environmental chambers set at constant 15, 20, 25, 30, or 35°C (≈65% RH, 16: 8 h L:

D photoperiod). A sheet of moist paper towel was placed in the emergence containers and re-wetted as needed to provide humidity. This procedure was repeated (replicated) until a total of 10 *S. galinae* broods (parasitized hosts) were observed for each temperature. Emergence containers were checked daily for parasitoid emergence. The number of female and male parasitoids emerged was tallied for each date of emergence. Since no parasitoids emerged from either 15 or 35°C these temperatures were excluded from data analyses.

Temperature-dependent reproductive biology of *S. galinae*

The objective of this experiment was to characterize the effect of constant ambient temperature (15, 20, 25, 30, and 35°C) on the longevity, survival, fertility, and parasitism of *S. galinae*. For each temperature, 15 female and male pairs (replicates) of newly (≤24h) emerged *S. galinae* parasitoids were isolated in individual rearing cages (≈16 cm length x 6 cm dia. polycarbonate tubing fitted with plastic caps, US Plastics) and provided excess 7 wk-old (3^{rd}-4^{th} instar) emerald ash borer host larvae on a constant basis throughout their lifetimes (until female mortality). All cages were maintained inside environmental chambers set at 15, 20, 25, 30, or 35°C (≈65% RH, 16: 8 h L: D photoperiod) for the duration of the experiment. A HOBO data logger (Onset Computer Co., Bourne, MA) was used to monitor the temperature and RH inside of each chamber. Typically, 3 — 4 emerald ash borer hosts were provided in tropical ash logs, which were changed twice per week (every Tuesday morning and Friday afternoon). Female and male parasitoids were monitored daily for mortality. After the parasitoid exposure period, logs were removed and placed in emergence

containers (as above) and incubated at 26 ± 1°C for 2 wk. After 2 wk, logs were dissected and larvae scored for parasitism. Parasitized larvae were easily observed because of the presence of silken cocoons spun by the fifth instar parasitoid larvae prior to pupation. The number of parasitized larvae, as well as the number of cocoons in each larval gallery, was tallied for each log (exposure period). Clover honey was *ad libitum* streaked onto screens on the rearing cages to provide a food (energy) source for the parasitoids throughout the experiment.

In addition to the reproductive (ovipositing) pairs, 15 female and 15 male parasitoids were held in rearing cages, as above, for each temperature and provided honey but were never offered emerald ash borer hosts. Cages were checked daily and the date of natural mortality for each female and male parasitoid was recorded. Longevity was recorded for both females and males, and weekly survivorship curves for females were estimated for each temperature to compare the life expectancy of ovipositing female parasitoids (exposed with hosts) to those females deprived of hosts.

Temperature-dependent life table for *S. galinae*

A temperature-dependent adult life table for *S. galinae* was constructed using equations from Southwood and Henderson (2000). A life table for an insect population is based on the female fertility schedule, which considers the number of daughters born (m) per female at age x (m_x), and the probability of survival (l) at age x (l_x). To calculate m_x values for *S. galinae*, the weekly fertility of each ovipositing female was estimated by totaling the number of cocoons (progeny) observed from each of the two exposure periods for each week of life. This number was then multiplied by the female

sex ratio (2.5: 1) of *S. galinae* progeny typically emerging from 7 wk-old emerald ash borer hosts reared in tropical ash logs, as determined in Chapter 1. To obtain l_x, the weekly survival of each female was estimated for each week of life. Maintaining constant time intervals, female net reproductive rates (R_0) were estimated using the equation $R_0=\Sigma\ l_x m_x$. Generation times (T_c) were directly estimated by adding the immature development time (from egg to adult emergence) to the female pre-oviposition period for each temperature. The innate capacity for increase (r_c), an approximation of the intrinsic rate of increase (r_m), may be applied to insect populations with discrete generations and high R_0 values (Laughlin 1965). This value describes the exponential growth potential of a population with a stable-age distribution under defined environmental conditions and is calculated as $r_c=\log_e(R_0)/T_c$. The finite rate of increase ($\lambda=e^r$) is the constant factor by which a population multiplies per generation. Population doubling time ($T_d=\log_e(2)/r_m$) is the amount of time required for the population to double in size.

Data analysis

The effect of temperature on immature development time and sex ratio of *S. galinae* were analyzed using one-way analyses of variance (ANOVA), followed by Tukey-Kramer's test for honestly significant differences (HSD) among mean values. Female pre-oviposition periods were calculated as the number of days elapsed from the date of adult emergence to the first exposure date exhibiting parasitism. Parasitism rates were estimated for each exposure period by dividing the number of parasitized hosts by the total number of available hosts in the log; no hosts were parasitized at

35°C so this temperature was excluded. The longevities of both females and males exposed to emerald ash borer hosts, and females and males maintained without hosts (host-deprived) were estimated by counting the number of days elapsed from adult emergence until mortality. Survivorship curves for both ovipositing and host-deprived female parasitoids were estimated using the nonparametric Kaplan-Meier survival analysis platform. The lifetime fertility of ovipositing females was calculated as the total number of progeny (no. of cocoons) observed from parasitized hosts throughout the life span. Effects of temperature on rates of parasitism, female pre-oviposition period, lifetime fertility, and parasitoid longevity were analyzed using one-way ANOVAs followed by the Tukey-Kramer HSD test to compare differences among the mean values. All analyses were carried out using JMP 10.0.1 (SAS Institute 2012).

Results

Temperature-dependent development of *S. galinae*

Temperature influenced immature development of *S. galinae*. When held at 15°C, parasitoid eggs hatched and larvae developed to fifth instars (cocoons), but progeny did not develop beyond this stage; at 35°C eggs became desiccated and did not hatch. Adult emergence occurred at 20, 25, and 30°C, and ANOVA revealed significant differences in development time among these temperatures ($F=53.4$, $df=2$, $P<0.0001$). The time it took for development from egg to adult was 24.8, 31.5, and 38.0 d for 30, 25, and 20°C, respectively (Table 3). Temperature did not significantly affect the sex ratio of parasitoid progeny emerging from these sticks ($F=2.4$, $df=2$, $P=0.1072$; Table 3).

Temperature-dependent reproductive biology of *S. galinae*

Except for 35°C, *S. galinae* parasitized emerald ash borer at all other constant ambient temperatures (15, 20, 25, 30°C), though the rate of parasitism was significantly higher at 25°C (35%) than for the other temperatures ($F=18.6$, $df=4$, $P<0.0001$; Figure 5). The mean pre-oviposition period was 16.4 d at 15°C, which was significantly longer than for the other temperatures ($F=13.7$, $df=3$, $P<0.0001$; Table 3). For female parasitoids exposed to emerald ash borer hosts throughout their lifetimes, mean longevity was significantly longer at 15°C (64.2 d) than for the other temperatures ($F=23.2$, $df=4$, $P<0.0001$; Table 3); the mean longevity of their male counterparts was also significantly longer at 15°C (60.9 d) than for the other temperatures ($F=8.8$, $df=4$, $P<0.0001$; Table 3). For host-deprived parasitoids mean longevity of females was significantly longer at 15°C (103.4 d) than for the other temperatures ($F=24.7$, $df=4$, $P<0.0001$); mean longevity of males was also significantly longer at 15°C (108.1 d) than for the other temperatures ($F=26.9$, $df=4$, $P<0.0001$; Table 3). Survival of females was significantly influenced by both temperature (Log-rank test, $\chi^2=107.8$, $df=4$, $P<0.0001$) and host deprivation (Log-rank test, $\chi^2=6.99$, $df=1$, $P=0.0082$). For ovipositing females, median survival was 11.5 wk (95% CI: 6 — 12 wk), 8 wk (95% CI: 7 — 9 wk), 7 wk (95% CI: 5 — 7 wk) 6.5 wk (95% CI: 5 — 8 wk) and 1 wk (95% CI: 0.5 — 2 wk) at 15, 20, 25, 30, and 35°C respectively (Figure 6). For host-deprived females, median survival was 15 wk (95% CI: 9 — 16 wk), 10.5 wk (95% CI: 4 — 13 wk), 9.5 wk (95% CI: 6 — 11 wk), 9.5 wk (95% CI: 3 — 11 wk), and 1 wk (95% CI: 0.5 — 2 wk) at 15, 20, 25, 30, and

35°C, respectively (Figure 6). Lifetime fertility was significantly impacted by temperature ($F=10.4$, $df=4$, $P<0.0001$), with females living at 25°C producing a mean of 42.6 progeny throughout their lifetimes, while females held at 35°C did not produce any progeny (Table 3). However, no significant differences were detected for lifetime fertility of females between 15 and 30°C (Table 3).

Temperature-dependent life table for *S. galinae*

For each temperature, female fertility schedules ($l_x m_x$) were constructed to enable the calculations of R_0. Of the temperature treatments where emerald ash borer were parasitized (15, 20, 25, and 30°C), the highest R_0 value (26.9) was at 25°C, while R_0 was lowest (16.5) at 30°C. Generation time (T_c) was longest (45.1 d) at 20°C and least (30.3 d) at 30°C (Table 2). At 15°C all progeny went into diapause and at 35°C progeny did not develop, so T_c could not be calculated for these temperatures. Thus, capacity for increase (r_c), which accounts for both T_c and R_0, could only be calculated for the temperatures where T_c and R_0 were calculated. At 20°C r_c was 0.07 and at both 25 and 30°C it was 0.09 (Table 4). The finite rate of increase (λ) was 1.07 at 20°C and 1.09 at both 25 and 30°C. Population doubling time was 9.9 at 20°C and 7.7 at both 25 and 30°C (Table 4).

Discussion

Understanding how temperature impacts the reproductive and developmental biology of parasitoids used in biological control is important when estimating their establishment potential in newly invaded regions (Pilkington et al. 2014).

Additionally, identifying the thermal requirements that optimize development time and reproductive output is critical for developing effective mass-rearing protocols (Qiu et al. 2012). One of the most prohibitive components of any biological control program is costs associated with rearing the natural enemy. The results of this study show that the optimum temperature for rearing *S. galinae* in the laboratory appears to be around 25°C (≈65% RH, 16L: 8D photoperiod). This temperature produced the highest R_0, which is a good estimator of fitness (Kingsolver and Huey 2008). Additionally, r_c was also greatest at 25°C; this metric accounts for generation time, survival, and fertility. Rearing *S. galinae* at 25°C should, therefore, boost parasitoid stock in the shortest time frame.

When late instar emerald ash borer larvae were exposed to adult *S. galinae* females at a parasitoid: host ratio of 6: 1 for 24 h, and parasitized hosts subsequently moved to temperatures of 15, 20, 25, 30, and 35°C with constant humidity and photoperiod, adult emergence occurred only at 20, 25, and 30°C. The period for immature development was significantly longer at 20°C (≈38 d) than at 25°C (≈32 d) and 30°C (≈25 d), though the sex ratio of adult progeny was not affected by temperature. A recent study by Duan et al. (2014) characterized the development of *S. galinae* at 25°C. In this study the authors found that adult emergence was completed ≈29 d after oviposition, however they used artificially inserted 'J-larvae' (mature 4th instar, non-feeding, J-shaped larvae) to facilitate daily observations of the immature progeny; J-larvae are the largest (in biomass) hosts used by *S. galinae*. Thus it is not surprising that adult emergence was completed several days earlier at 25°C in their

study (29 d compared to 32 d with actively feeding 4th instar larvae) when J-larvae were used as hosts because developmental rates for some parasitoid species increase with a greater availability of host resources (Godfray 1994).

In the present study, no *S. galinae* adults emerged when the immature progeny was exposed to constant ambient 15 and 35°C. For the 35°C treatment, when emerald ash borer host larvae were removed from the sticks maintained and placed in water in a petri dish for 24 h and then dissected under a microscope, several *S. galinae* egg clutches were found on the cuticle of hosts. However all of the eggs appeared desiccated and no parasitoid larvae hatched. At 15°C, parasitoids developed only to 5th instars (cocoons) where it was suspected that progeny entered diapause. To confirm this, parasitoid cocoons were removed from 15°C and either moved to a lower temperature (≈2°C), or maintained at 15°C for three months, then placed in an environmental chamber set at 25°C where adult emergence then occurred. This finding was intriguing; when *S. galinae* adults were held at 15°C for their lifetimes and their progeny (from parasitized hosts) moved to 26°C for emergence, nearly all progeny emerged. However, when immature parasitoids were maintained at 15°C beginning as eggs, progeny entered diapause as 5th instar larvae (cocoons). Thus it appears that *S. galinae* diapause is triggered by the thermal experience of the immature progeny, rather than by that of the parents. Additional studies are now being conducted to experimentally evaluate the conditions controlling diapause in *S. galinae* (J.J. Duan and J. Gould, personal communication).

There was an obvious effect of temperature on the reproductive parameters of *S. galinae*. While all temperatures (15, 20, 25, and 30°C) supported emerald ash borer parasitism except 35°C, the rate at which larvae were parasitized was significantly greater at 25°C than for the other temperatures. Of those temperatures where parasitism occurred, lifetime fertility peaked at 25°C (≈43 progeny per female), while females held at 30°C produced the fewest offspring (≈24 progeny per female), although the differences were not statistically significant. Longevity and survival, which also affect estimates of R_0 and r_c, were inversely associated with temperature. Whether exposed with or without emerald ash borer larvae, both male and female parasitoids lived longest at 15°C, and life expectancy decreased as temperature increased.

When exposed with hosts, ovipositing *S. galinae* females lived ≈64 d on average; when deprived of hosts they lived over 100 d. Survival analyses revealed that host-deprivation significantly lengthened the life expectancy of female parasitoids in comparison to females that oviposited on hosts during their lifetimes. In synovigenic species, females are known to resorb their eggs after extended periods of host deprivation (Jervis et al. 2005). This action conserves energy until hosts are present. Thus, it is not surprising that females exposed without hosts lived longer, at all temperatures except 35°C, than females needing energy to parasitize hosts. Survivorship curves for female *S. galinae* were a typical type I at all temperatures except 35°C, where it was an extreme type II. Survivorship curves display how the risk of mortality relates to time. With type I survivorship, mortality risk is lower early

in life and increases with time, whereas mortality risk is constant with a type II curve (see Jervis et al. 2005).

There was a pre-oviposition period for female parasitoids living at all temperatures, and the temperature-dependent fertility curves for *S. galinae* (see Figure 6) were typical of a synovigenic parasitoid (see Calvo et al. 2013). At 15°C, the mean pre-oviposition period was longest (16 d), while at 25°C it was shortest (5 d). In contrast to pro-ovigenic species, where egg loads are essentially maximized at birth, synovigenic species require time for ovigenesis. Temperature is more likely to influence synovigenic species because ovigenesis is a metabolic function dependent on temperature (Jervis et al. 2005). For synovigenic species the general trend is for reproduction to occur earlier in life with increasing temperature, although at high temperatures this trend may not hold (Jervis et al. 2005).

One major advantage of *S. galinae* in the context of the current emerald ash borer biological control program is that it appears to be more cold-hardy than *S. agrili*. The results from this study show that *S. galinae* are able to parasitize emerald ash borer even when the ambient temperature is 15°C, although constant 15°C induced diapause in immature progeny. Currently, the northernmost distribution of emerald ash borer in North America is Houghton, MI, where the annual mean mid-summer (June-Aug.) temperature is ≈19°C (US Climate Data). In the present study, when *S. galinae* lived near this temperature (20°C), its R_0 was nearly 26 and its capacity for increase (r_c) was 0.07, while its net reproductive rate (R_0) was nearly 18 when females were

maintained at 15°C, indicating that *S. galinae* should be able to reproduce in these northern regions.

Gould et al. (2011) calculated an r_c of 0.09 for *S. agrili,* but when reared using daily fluctuating temperatures of 20 — 25°C. They also noted, however, that in China *S. agrili* appears to be most active at 30°C. Although behavior was not experimentally investigated in this study on *S. galinae*, daily observations were conducted to determine exact times of mortality. During these observations, it was evident that both female and male parasitoids living at 30°C were less active than those living at 20 and 25°C. Additionally, at 35°C, where *S. galinae* females lived only an average of 10 d, no oviposition behavior was observed, and both males and females were most often noticed standing on the bottom of the cages with their wings outstretched. Upon death, the ovaries of these females were dissected but no eggs were observed. It is likely that parasitoids exposed to this high temperature for extended periods became stressed and no energy resources were allocated toward mating or female egg maturation. Furthermore, it is interesting to note that the *S. galinae* pre-oviposition period was, on average, slightly longer at 30°C than at 25°C, although the difference was not statistically significant. Taken together, the findings that 30°C required a longer pre-oviposition period than 25°C, that lifetime fertility and R_0 both were least at 30°C, and that reproductive activity ceased altogether at 35°C, indicate that 30°C may be outside the optimal thermal window for *S. galinae* reproduction. Additional studies should confirm if this is true. Furthermore, studies about the winter cold tolerance of *S.*

galinae should be a priority. Information from such studies would be critical in determining the establishment potential of *S. galinae*.

If 30°C is indeed outside the optimal thermal window for *S. galinae* reproduction, this would have implications for its use in emerald ash borer biological control. Along the US latitudinal gradient, Cincinnati, OH, is the northernmost city in the Midwest and eastern regions (latitude of 39.1°N) of the US with annual summer temperatures above 30°C (US Climate Data). In this and more southern regions, where reduced rates of parasitism, survival, or fertility will be likely when temperatures are above 30°C, consequently resulting in restricted biological control. Conversely, in more northern regions, *S. galinae* may be better suited for introduction. Lelito (2013) explained that, due to its lack of success establishing in northern regions, no further releases of *S. agrili* are planned north of 40°N latitude; hopefully *S. galinae* will be able to establish where *S. agrili* thus far has not. There are a number of elements, however, that influence insect performance in the field, and how *S. galinae* is able to adapt to local climates in North America will be an important area of research.

Table 3. *Spathius galinae* development time, sex ratio, adult pre-oviposition period, longevity, and fertility as influenced by ambient temperature.

	Temperature (°C)[a]				
	15	20	25	30	35
Development time (egg to adult)[b]	-*	38.0 ± 0.9a	31.5 ± 1.0b	24.8 ± 0.9c	-**
Emerged progeny female sex ratio	-	1.5 ± 0.2a	1.9 ± 0.1a	1.9 ± 0.1a	-
Adult pre-oviposition period[b]	16.4 ± 1.41a	7.13 ± 1.40b	5.33 ± 1.41b	5.54 ± 1.51b	-
Female longevity (with hosts)[b]	64.2 ± 3.9a	53.7 ± 4.0ab	45.1 ± 4.8b	43.2 ± 3.9b	9.9 ± 4.4c
Male longevity (with hosts)[b]	60.9 ± 6.5a	51.0 ± 6.6ab	42.6 ± 7.1b	32.7 ± 6.1b	7.2 ± 7.9c
Female longevity (host deprived)[b]	103.4 ± 7.2a	69.5 ± 6.7b	67.4 ± 6.7b	54.8 ± 6.7b	8.8 ± 6.7c
Male longevity (host deprived)[b]	108.1 ± 6.3a	64.6 ± 6.3b	58.0 ± 6.2b	55.6 ± 6.2b	5.2 ± 7.9c
Lifetime fertility	26.3 ± 4.7a	37.1 ± 4.7a	42.6 ± 4.7a	24.3 ± 4.7a	0.0 ± 0.0b

* All larvae entered diapause in the cocoon stage

** Eggs desiccated prior to hatching

[a] Mean (± SE) values with different letters are significantly different

[b] Values are in days.

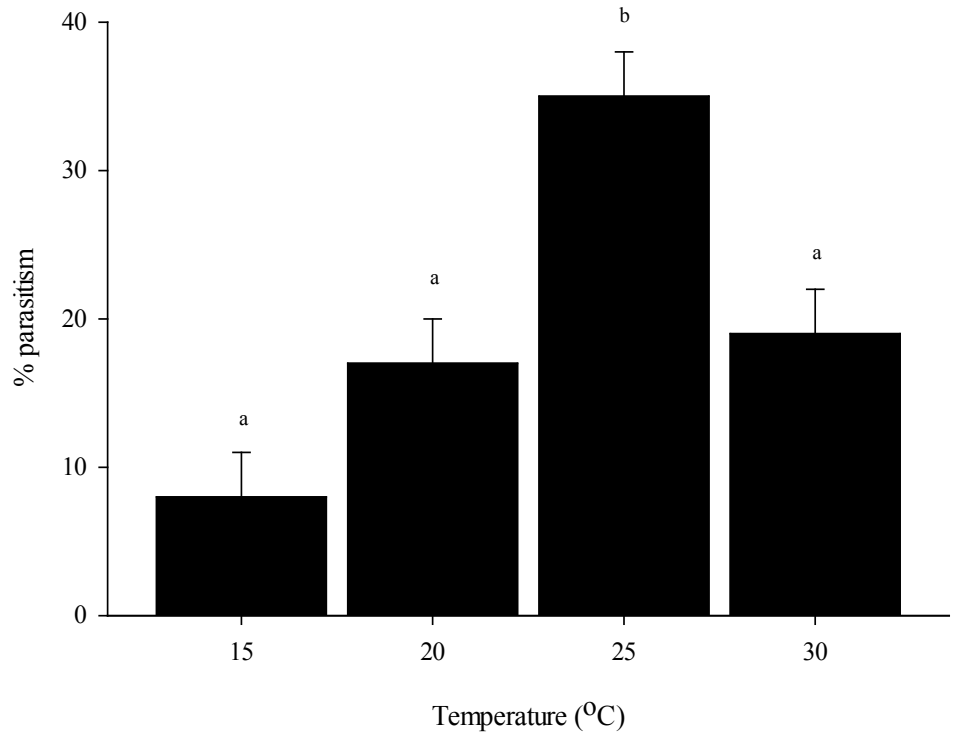

Figure 5. Mean (± SE) parasitism rate of late instar EAB larvae by *Spathius galinae* as influenced by ambient temperature. Bars with different letters are significantly different (α=0.05).

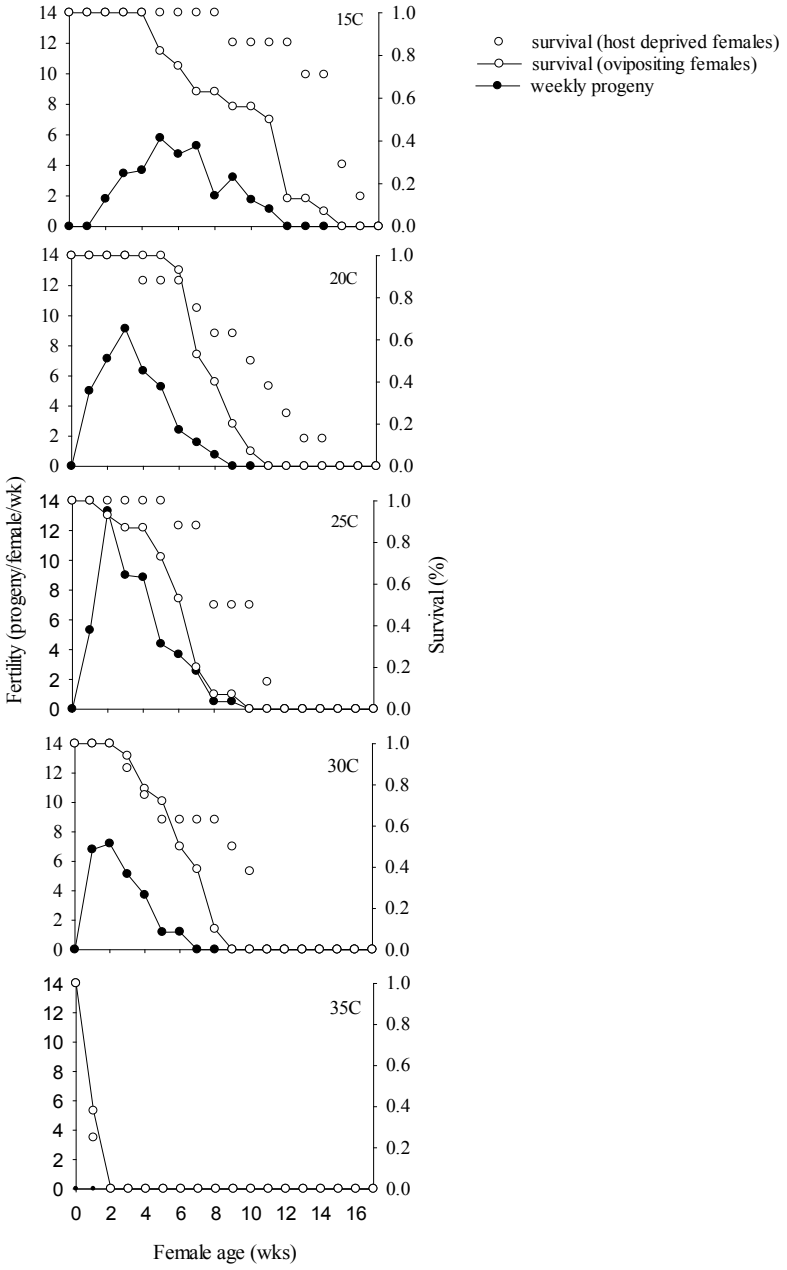

Figure 6. Weekly survivorship and fertility of *Spathius galinae* as influenced by ambient temperature. Solid lines with black circles are the mean number of progeny (male and female) produced by a single ovipositing female for each week of life. Solid lines with white circles are the life expectancy of ovipositing females; dotted lines with white circles are the life expectancy of host-deprived females

Table 4. *Spathius galinae* life table parameters as influenced by ambient temperature.

Temp. (°C)	R_0	T_c	r_c	λ	T_d
15	17.80	0.00	0.00	0.00	0.00
20	25.71	45.13	0.07	1.07	9.90
25	26.89	36.83	0.09	1.09	7.70
30	16.45	30.34	0.09	1.09	7.70
35	0.00	0.00	0.00	0.00	0.00

Chapter 4

INFLUENCE OF HOST AND PARASITOID GROUP STRUCTURE ON FITNESS OF *SPATHIUS GALINAE*

Introduction

The production of insect natural enemies for biological control can be expensive (Heinz 1998, Parrella et al. 1992), and the economic significance of inefficient mass-rearing is substantial (Irvin and Hoddle 2006). A goal of insect mass-rearing should always be to develop techniques that maximize fitness while minimizing costs associated with production (Singh 1982). Often, when rearing natural enemies with inherently female-biased sex ratios, such as haplodiploid hymenopteran parasitoids, producing as many females as possible while assuring only enough males to inseminate females is a target because females are what provides control in the field and boosts colonies in culture (Ode and Heinz 2002, Ode and Hardy 2008).

Haplodiploid hymenopteran parasitoids may adjust both the quantity and gender of their offspring according to a number of variables, including host quality, environmental conditions, prior experience, and crowding. In mass-rearing, tendencies toward male-biased sex ratios may exist when too many ovipositing (foraging) females are exploiting the same patch of hosts in rearing cages (Irvin and Hoddle

2006, Ode and Hardy 2008). This occurs due to a phenomenon known as local mate competition (LMC), which was first outlined by Hamilton (1967). LMC predicts that female parasitoids maximize fitness by increasing investments in male progeny when the number of foraging females visiting a patch increases, because more sons would have a greater likelihood of inseminating females from unrelated lineages and thereby adding to the fitness of their own lineage (Werren 1980, 1983, Godfray 1994). In addition, female crowding may lead to superparasitism (multiple parasitisms of the same host by conspecific individuals), which, in gregarious species, may lead to larger broods of smaller individuals, also affecting fitness (Waage et al. 1985).

Some parasitoid species, however, increase fitness in the presence of other foraging females due to a type of behavior known as socially facilitated egg-laying (oviposition). Socially facilitated oviposition holds that the number of offspring produced per parent female increases when there are other nearby females foraging for hosts (Prokopy and Duan 1998, Prokopy and Reynolds 1998). For these types of parasitoids, more progeny are produced when females are exploiting hosts in groups rather than individually. Identifying the factors influencing fitness is important for economically rearing high-quality biological control agents, which makes their purchase more affordable.

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a rapidly spreading invasive pest that threatens the ecological functions and services of ash (*Fraxinus* spp.) tree-based forest ecosystems in North America (Herms and McCullough 2014). Shortly after its detection, a classical biological control program

for emerald ash borer was initiated involving the release of three hymenopteran parasitoids from the pest's native range (northeast Asia): the egg parasitoid *Oobius agrili* Zhang and Huang (Encrytidae), the larval endoparasitoid *Tetrastichus planipennisi* Yang (Eulophidae), and the larval ectoparasitoid *Spathius agrili* Yang (Braconidae). While biological control attributed to these parasitoids has been documented (Duan et al. 2013b), high production costs, especially for larval parasitoids, has limited their mass-production (Lelito et al. 2013). To date, no artificial diet has successfully been developed to consistently produce high quality emerald ash borer larvae suitable for parasitoid rearing. Recently, an alternative host plant-based method for rearing emerald ash borer was developed (Duan et al. 2011, 2013a) that holds promise for reducing parasitoid production costs; nevertheless, minimizing costs associated with rearing emerald ash borer parasitoids is necessary for increasing the affordability of biological control.

Spathius galinae Belokobylskij and Strazenac (Hymenoptera: Braconidae) is a recently discovered gregarious idiobiont ectoparasitoid of late instar emerald ash borer larvae (Duan et al. 2014). To complement the current emerald ash borer biological control program, releases of *S. galinae* are anticipated for 2015 (Gould and Duan 2013). To assist its mass-rearing, the effects of parasitoid: host ratio and parasitoid and host group size (group structure) on *S. galinae* fitness were investigated in laboratory experiments.

Materials and Methods

Emerald ash borer larvae

Larvae used in the following experiments were reared until 7 wk of age in small (9 cm length x 1-1.5 cm diameter) evergreen (tropical) ash (*Fraxinus uhdei* [Wenzig] Lingelsh) sticks (as in Chapter 2) or in large (25cm length x 3-4 cm diameter) tropical ash sticks, using methods described in Duan et al. (2011, 2013a). Briefly, emerald ash borer eggs, freshly oviposited on unbleached coffee filter paper (HomeLife, Eden Prairie, MN), were placed individually against the surface of tropical ash sticks and then tautly wrapped to the sticks with Parafilm (BEMIS, BEMIS flexible packaging, Neenah, WI). Tropical ash sticks (infested with emerald ash borer eggs) were placed on water-saturated floral foam bricks (OASIS, Smithers-Oasis Company, Kent, OH) and maintained in AR-360 environmental growth chambers (Percival Scientific, Perry, IA) set at 25 ± 1^{o}C, 65% RH, and 16:8 h L:D photoperiod for 7 wk until ready for parasitoid exposure. Duan et al. (2013a) found that emerald ash borer larvae reach late (3rd – 4th) instars ≈7 weeks after oviposition when reared under the above environmental conditions. Prior to parasitoid exposure, sticks were checked for the presence of emerald ash borer hosts by shaving back a thin strip of bark to look for frass in galleries made by the larvae; sticks without larvae (or if larvae appeared unhealthy) were discarded.

Parasitoids

All *S. galinae* used throughout the study were $F_{10\text{-}12}$ progeny from a founder colony collected near Vladivostok, Russia (Russian Far East) in the fall of 2010 (Duan et al. 2012). All female *S. galinae* used throughout the study were naïve, 2-3 wk-old, and gravid. Upon emergence, parasitoids were maintained in ventilated acrylic cylinders at a ≈1: 2 male: female ratio and provided clover honey *ad libitum* (see chapter 1 for similar description).

Effect of parasitoid: host ratio on host utilization and fitness of *S. galinae*

The objective of this experiment was to assess the impact of parasitoid: host ratio on the host utilization rate (probability of parasitism) and critical fitness parameters (brood size, sex ratio, and anatomical measurements of progeny) of *S. galinae*. Four different parasitoid: host ratio treatments were created (1: 1, 2: 1, 4: 1, and 8: 1) by varying the number of foraging female parasitoids in rearing cages, maintaining constant host density (one larva). For each treatment, a single tropical ash stick containing one 7 wk-old emerald ash borer larva (3^{rd}-4^{th} instar) was placed in a rearing arena (≈16 cm length x 6 cm diam. polycarbonate tubing fitted with plastic caps, US Plastics) and randomly assigned to one of the treatments. Either 1, 2, 4, or 8 female *S. galinae* were presented to hosts using a fine camel paintbrush (Simply Simmons 25 Round, Daler-Rowney, Cranbury, NJ) to gently coax them into exposure arena. Thirty-two replicates of each treatment were performed using a randomized block design, where the same batch of parasitoids was used for all treatments for each

replicate (block). After a 7-d exposure period, parasitoids were removed from rearing cages and tropical ash sticks (containing hosts, still in rearing cages) were placed in a climate-controlled room set at 26°C ± 1°C (≈65% RH, 16: 8 h L: D photoperiod) until parasitoid progeny emergence. Emerged *S. galinae* progeny (if any) were counted and the sex of the individuals recorded for each treatment. For treatments producing parasitism, one female and one male parasitoid were randomly selected and placed in a vial of 95% ethyl alcohol for later measurement of anatomical fitness markers (length of body, left hind tibia, and female ovipositor), as described in Chapter 1.

Effect of parasitoid and host group size on *S. galinae* parasitism and progeny production

The objective here was to investigate the effects of varying parasitoid and host group sizes (using a constant size rearing arena and 1: 1 parasitoid: host ratio) on parasitism rate, the number of progeny produced per female parasitoid, and sex ratio. Four treatments were used: 1-1, 5-5, 10-10, and 20-20 parasitoids-hosts. Treatments consisted of 1, 5, 10, or 20 female *S. galinae* exposed to the corresponding number of emerald ash borer larvae (1, 5, 10, or 20) in a constant size rearing arena. Rearing arenas consisted of a 20 x 10 cm ventilated cylindrical cage tightly fastened to the inside lip of a 1 liter white polypropylene cup (Berry Plastics Co., Evansville, IN). Ten replicates of each treatment were performed, except for the 1-1 parasitoid-host treatment where 13 replicates were performed, using a similar design as above. Because different numbers of host larvae were required for each treatment, different numbers of emerald ash borer eggs were used to infest the tropical ash sticks. To

account for a low level of larval mortality, 7, 12, and 25 eggs were used to obtain 5, 10, and 20 host larvae, respectively. Because rearing only one emerald ash borer larva in large sticks (as used for the 5-5, 10-10, and 20-20 parasitoids-hosts treatments) often results in larval mortality (due to plant resistance), larvae for this treatment (1-1 parasitoid-host) were reared in small sticks (as described for the parasitoid: host ratio experiment). For each 1-1 parasitoid-host treatment, three clean (without emerald ash borer larvae) tropical ash sticks were supplied in the rearing arena in order to maintain similar host-plant surface areas as for the treatments where large sticks were used. For all treatments, after the 7 d exposure period, parasitoids were removed from rearing arenas and tropical ash sticks (containing hosts, still in rearing arenas) were placed in a climate-controlled room for parasitoid emergence, as described above.

Data Analysis

For the parasitoid: host ratio experiment, a nominal logistic regression was used to analyze the relationship between the number of foraging female parasitoids and the host utilization rate (probability of parasitism). Brood size was estimated for each trial as the total number of male and female parasitoids emerging from the host larva; sex ratios are reported as the ratio of female to male offspring in the emerged progeny. The anatomical estimates of progeny fitness (measured as length of body, left hind tibia, and female ovipositor) were recorded for each of 25 females. Brood sizes, sex ratios and estimates of progeny fitness were first log-transformed and then subjected to one-way analyses of variance (ANOVA) to estimate the mean values for

all trials (replicates), followed by Tukey-Kramer's HSD test to compare the mean values.

For the parasitoid and host group size experiment, parasitism rates were determined for each exposure assay by dividing the total number of parasitized hosts by the number of available hosts in the log at the time of parasitoid exposure. The number of progeny produced per female parasitoid in each assay was determined by dividing the total number of progeny produced by the number of female parasitoids used in the assay. Sex ratio of progeny is reported as the ratio of female to male parasitoids produced from each replicate stick. Prior to analyses, parasitism rates were square-root arcsine transformed and the number of progeny per female parasitoid and progeny sex ratio were log-transformed for normality. One way ANOVAs were used to determine the means of all treatments followed by Tukey-Kramer's HSD test to look for significant differences among the mean values. All analyses were carried out using JMP 10.0.1 (SAS Institute 2012).

Results

Effect of parasitoid: host ratio on host utilization and fitness of *S. galinae*

When a single emerald ash borer larva was presented as a host to varying numbers foraging female parasitoids, the probability of host parasitism significantly increased when there were more females (Likelihood ratio X^2=19.48, df=1, P<0.0001), with the probability of host parasitism being 0.34, 0.37, 0.68, and 0.79 when 1, 2, 4, and 8 females were exposed, respectively (Figure 7). The logistic model was appropriate to describe the relationship between probability of parasitism and the

number of foraging female parasitoids (Lack of Fit: X^2=3.53, df=2, P=0.17). However, the number of female parasitoids did not significantly impact brood size (F=0.11, df=3, P=0.95), sex ratio (F=0.5, df=3, P=0.7), or the anatomical estimates of progeny fitness (Table 5).

Effect of parasitoid and host group size on *S. galinae* parasitism and progeny production

Parasitoid and host group size significantly affected both the rate of parasitism by *S. galinae* (F=16.9, df=3, P<0.0001) and the number of progeny (male and female) produced per female parasitoid (F=28.2, df=3, P<0.0001). When group size was 1-1, the mean percentage of larvae parasitized was 45.6, while the mean percentage parasitism was 88.1 when group size was 20-20 parasitoids-hosts (Table 6). The mean number of progeny produced per female parasitoid was 3.8 when group size was 1-1, while it was 7.0 and 6.1 when group size was 10-10 and 20-20 parasitoids-hosts, respectively. Parasitoid and host group size did not significantly affect the sex ratio of progeny (F=1.0, df=3, P=0.39; Table 6).

Discussion

Identifying factors influencing natural enemy fitness is a key element of successful biological control, for both mass-rearing purposes and for understanding performance in the field. When only one late instar emerald ash borer larva was provided as a host to different numbers of foraging *S. galinae* females in a rearing arena, host parasitism rates were positively associated with increasing parasitoid: host ratios. This finding was expected; there is usually a greater chance of parasitism when

more parasitoids are available for oviposition. However, too many females simultaneously foraging for hosts in a rearing arena may adversely affect progeny sex ratios (i.e. too many males), which reduces rearing efficiency and inflates production costs (Waage et al. 1985, Irvin and Hoddle 2006). Hamilton's (1967) LMC model asserts that the proportion of male offspring produced will be higher when the density of females exploiting a host patch is higher. This model, however, is intended to describe a situation where multiple hosts in a close proximity (host patch) are being exploited. In the parasitoid: host ratio experiment, where only one host was available to parasitoids, no significant differences were observed in progeny sex ratio as a result of increasing the parasitoid: host ratio.

Superparasitism—multiple parasitisms of the same host by conspecific parasitoids—is a common phenomenon in Hymenoptera (Fellowes et al. 2005). Although superparasitism may result in more progeny per host, fitness of individuals in the progeny may be lessened. In a similar study to the one reported in this chapter, Duan and Oppel (2012) investigated the effects of parasitoid and host group structure for *T. planipennisi*, a gregarious koinobiont parasitoid of late instar emerald ash borer larvae. The authors found that significantly more progeny were produced per host when parasitoid: host ratio increased, and although they did not explicitly point to superparasitism in their study, *T. planipennisi* females were often observed aggregating near hosts, with multiple females appearing to oviposit in the same host (J.J. Duan, personal communication). Duan and Oppel (2012) also found that their anatomical estimates of *T. planipennisi* progeny fitness (length of female ovipositor

and left hind tibia) were inversely related with increasing the parasitoid: host ratio. It was concluded that higher ratios of female parasitoids to hosts adversely affected the fitness consequences of *T. planipennisi* progeny, and they advocated using a low (1: 1) parasitoid: host ratio when rearing this parasitoid.

In contrast to Duan and Oppel (2012), no significant differences in the number of *S. galinae* offspring produced per emerald ash borer host (brood size) were detected when parasitoid: host ratio increased. Additionally, no significant differences in the anatomical estimates of progeny fitness were detected when parasitoid: host ratio increased. These findings are attributed to the fact that *S. galinae* is an idiobiont parasitoid; while *T. planipennisi* is a koinobiont parasitoid, and its hosts continue to grow after oviposition, *S. galinae* paralyzes its hosts at the time of oviposition, permanently halting their growth. When emerald ash borer larvae feed in the cambium of ash trees they produce chewing vibrations that may be detected by parasitoids (Wang et al. 2010a, Ulyshen et al. 2010b, 2011). Therefore, while superparasitism might be expected with *T. planipennisi* (because hosts continue to feed), superparasitism might be prevented with *S. galinae*, because their hosts cease to feed, potentially preventing host detection by other parasitoids. Nevertheless, superparasitism has been observed in *S. galinae* (T. J. W., personal observation), but the extent to which it occurs is unknown, and any negative effects were undetected in this study.

When parasitoid and host group size (in a constant size rearing arena) varied, but at a constant 1: 1 parasitoid: host ratio, emerald ash borer parasitism rates were

positively associated with group size. When only one host was exposed to one female parasitoid, the rate of parasitism was ≈46%. However, when 20 hosts were exposed to 20 female parasitoids, parasitism was significantly higher (≈88%). Taking into consideration both the parasitoid: host ratio experiment and the parasitoid and host group size experiment, parasitism rates were never more than 50% when there was only one emerald ash borer larva and one female *S. galinae* exposed in a rearing arena. However, even when maintaining a 1: 1 parasitoid: host ratio, nearly 90% of emerald ash borer were parasitized when group size was 10-10 and 20-20 parasitoids-hosts. This finding indicates the possibility of socially facilitated oviposition behavior by *S. galinae*, and would have implications for mass-rearing.

Socially facilitated oviposition behavior occurs in other hymenopteran parasitoids and is when females produce more progeny in the presence of other conspecific females exploiting the same host patch, rather than alone (Ramadan et al. 1994, Prokopy and Duan 1998). This behavior may be an evolutionary adaptation increasing fitness in some species of nonsocial insects (Prokopy and Roitberg 2001). When females are overcrowded, however, fitness can be adversely affected (Prokopy and Roitberg 2001). While varying parasitoid and host group size did not influence *S. galinae* sex ratio, the average number of progeny produced per female parasitoid increased from ≈4 when group size was either 1-1 or 5-5, to 7 progeny per female when group size was 10-10 parasitoids-hosts. When group size was 20-20, the number of progeny produced per female significantly decreased to ≈6 when group size was 20-20 parasitoids-hosts.

In contrast to the parasitoid: host ratio experiment, where only one emerald ash borer larva was presented to varying densities of female *S. galinae*, and eight females were needed to achieve ≥75% parasitism, a 1: 1 parasitoid: host ratio was sufficient for achieving nearly 90% emerald ash borer parasitism when at least 10 female parasitoids were exposed to 10 host larvae. Neither sex ratio nor progeny fitness (as measured by body, tibia, and ovipositor length) was affected by differences in parasitoid and host group size. For mass-rearing, then, it appears that exposing emerald ash borer host larvae and *S. galinae* in a group setting (10 hosts: 10 parasitoids) will yield the highest parasitism and progeny.

These results have practical application when considering field releases of *S. galinae*. Here it is demonstrated that reproductive output (no. progeny per female parasitoid) is greater when parasitoids are present with hosts in medium (10-10 parasitoids-hosts) or high (20-20 parasitoids-hosts) densities, rather than in low (5-5 or 1-1 parasitoids-hosts) densities. Therefore, in the field, more parasitoid progeny may be produced per host when high densities of parasitoids are released into a patch with similarly high host densities. An important quality of *S. galinae*, however, is its ability to detect emerald ash borer hosts in low densities (prior to damaging infestations), and it will be critical to monitor how this parasitoid establishes in the field so as to understand the best practices for its release.

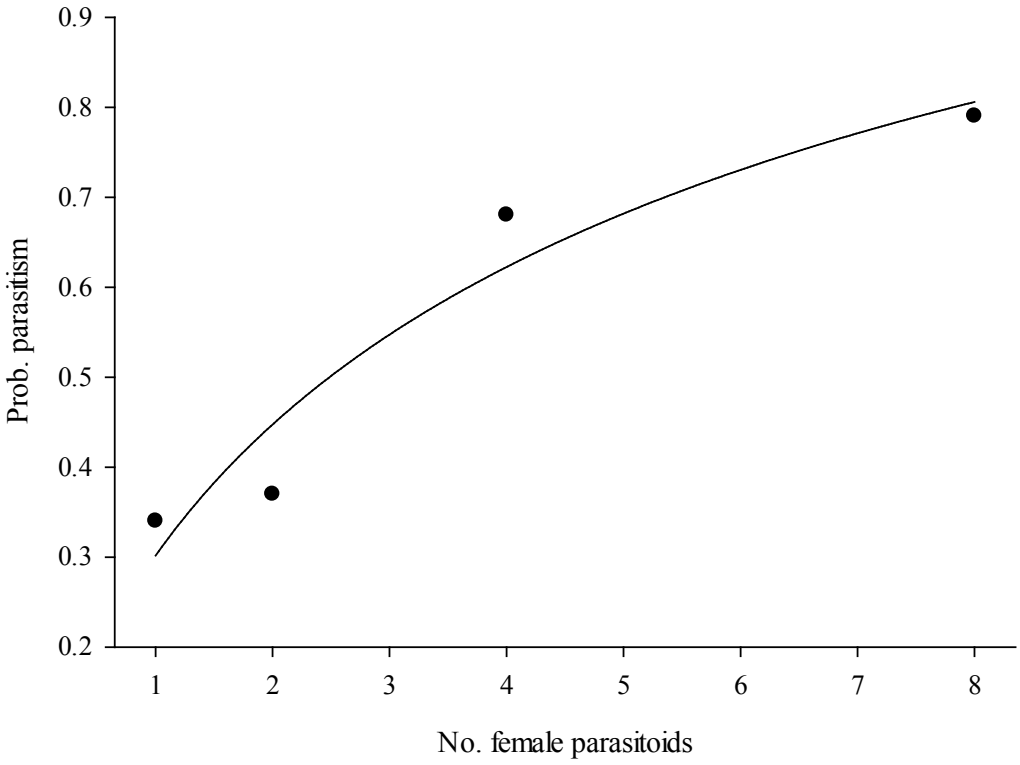

Figure 7. Probability of EAB host parasitism by *Spathius galinae* as influenced by the number of foraging female parasitoids. Black circles are the observed mean values for each treatment (1, 2, 4, and 8 females); the solid line is the prediction curve generated by the nominal logistic regression analysis ($y=a/1+(x/x_0)^b$).

Table 5. *Spathius galinae* brood characteristics and anatomical estimates of progeny fitness as influenced by the number of foraging female parasitoids (parasitoid: host ratio).

	Parasitoid: host ratio[a]			
	1: 1	2: 1	4: 1	8: 1
Brood size	6.3 ± 1.4a	5.8 ± 1.1a	6.4 ± 0.8a	6.6 ± 0.7a
Female sex ratio	1.6 ± 0.8a	2.0 ±0.7a	2.4 ± 0.5a	1.7 ± 0.4a
Body[b c]	3.8 ± 0.2a	3.5 ± 0.2a	3.4 ± 0.1a	3.5 ± 0.1a
Left hind tibia[b d]	1.2 ± 0.05a	1.0 ± 0.06a	1.0 ± 0.04a	1.0 ± 0.03a
Ovipositor[b e]	3.6 ± 0.2a	3.3 ±0.2a	3.2 ± 0.1a	3.2 ± 0.1a

[a] Mean (± SE) values with different letters are significantly different
[b] Lengths shown in mm
[c] F=1.2, df=3, P=0.34.
[d] F=2.2, df=3, P=0.11.
[e] F=1.0, df=3, P=0.40.

Table 6. *Spathius galinae* parasitism rates and progeny characteristics as influenced by parasitoid-host group rearing size.

	Parasitoid-host group size[a]			
	1-1	5-5	10-10	20-20
% Parasitism	45.6 ± 5.7a	81.0 ± 2.1b	85.5 ± 1.5bc	88.1 ± 1.1c
Progeny per female parasitoid	3.8 ± 0.5a	4.4 ± 0.3a	7.0 ± 0.2b	6.1 ± 0.1c
Female sex ratio	2.4 ± 0.4a	2.1 ± 0.2a	2.0 ± 0.1a	2.2 ± 0.1a

[a] Mean (± SE) values with different letters are significantly different

REFERENCES

Abell, K.J., J.J. Duan, L. Bauer, J.P. Lelito and R.G. Van Dreisch. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). Biol. Control. 63: 320-325.

Aukema, J.E., D.G. McCullough, B.V. Holle, A.M. Liebhold, K. Britton, and S.J. Frankel. 2010. Historical accumulation of nonindigenous forest pests in the continental United States. Bioscience 60: 886-897.

Baffoe, K.O., P. Dalin, G. Nordlander, and J.A. Stenberg. 2012. Importance of temperature for the biological control efficiency of the parasitoid *Perilitus brevicollis* (Hymenoptera: Braconidae) on *Salix*. BioControl. 57: 611-618. doi: 10.1007/s10526-012-9443-5

Bale J.S., G.J. Masters, I.D. Hodkinson, C. Awmack, T.M. Bezemer, V.K. Brown, J. Butterfield, A. Buse, *et. al.* 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Glob. Change Biol. 8: 1–16.

Bale, J.S. J.C. van Lentern, and F. Bigler. 2008. Biological control and sustainable food production. Phil. Trans. R. Soc. B 363: 761-776. doi: 10.1098/rstb.2007.2182

Belokobylskij, S. A., G. I. Yurchenko, J. S. Strazanac, A. Zaldı´var-Rivero´ n, and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius Nees* (Hymenoptera: Braconidae: Doryctinae) From the Russian Far East and South Korea. Ann. Entomol. Soc. Am. 105: 165-178.

Bezemer, T. M., and N.J. Mills. 2003. Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. Anim. Behav. 66(6): 1119–1128.

Boyd, I.L., P.H. Freer-Smith, C.A. Gilligan, and H.C.J. Godfray. 2013. The consequence of tree pests and diseases for ecosystem services. Science. 342: 823-832. doi: 10.1126/science.1235773

Calvo, F.J., J.D. Soriano, K. Bolckmans, and J.E. Belda. 2013. Host instar suitability and life history parameters under different temperature regimes of *Necremnus artynes* on *Tuta absoluta*. Biocontrol Sci. and Techn. 23(7): 803-815. doi: 10.1080/09583157.2013.795930

Cancino, J. and P. Montoya. 2006. Advances and perspectives in the mass rearing of fruit fly parasitoids in Mexico. Proc. of the 7th Int. Symp. on fruit flies of economic importance. Sept. 10-15, Salvador, Brazil. p. 133-142.

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.

Chabora, P.C. and D. Pimentel. 1966. Effect of host (*Musca domestica* Linnaeus) age on the pteromalid parasite *Nasonia vitripennis* (Walker). Can. Entomol. 98: 1226-1231.

Chamorro, M.L., M.G. Volkovitsh, T.M. Poland, R.A. Haack, and S.W. Lingafelter. 2012. Preimaginal stages of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae): an invasive pest on ash trees (*Fraxinus*). PLoS ONE. 7(3): e33185. doi:10.1371/journal.pone.0033185.

Charnov, E. L. 1982. The Theory of Sex Allocation. Princeton University Press. Princeton.

Charnov, E.L., R.L. Los-den Hartogh, W.T. Jones, and J. van den Assem. 1981. Sex ratio evolution in a variable environment. Nature. 289: 27-33.

Dixon, A.F.G., A. Honek, P. Keil, M.A.A. Kotela, A.L. Sizling, and V. Jarosik. 2009. Relationship between the minimum and maximum temperature thresholds for development in insects. Funct. Ecol. 23: 257-264. doi: 10.1111/j.1365-2435.2008.01489.x

Duan, J.J., M.D. Ulyshen, L.S. Bauer, J. Gould, and R. Van Driesche. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borer (Coleoptera: Buprestidae). Environ. Entomol. 39: 1513-1522.

Duan, J. J., T. Watt, and C.B. Oppel. 2011. An alternative host plant-based method for laboratory rearing of emerald ash borer to produce larval parasitoids for biological control. Pp. 107 – 108. In Parra G., D. Lance, V. Mastro, R. Reardon and C. Benedict (compiled): Proceeding of 2011 Emerald Ash Borer

National Research and Technology Development Meeting October 12 & 13, 2011 Wooster, Ohio. USDA Technology Transfer FHTET-2011-06, Raleigh, North Carolina.

Duan, J.J., G. Yurchenko, and R. Fuester. 2012. Occurrence of emerald ash borer (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far East. Environ. Entomol. 41(2): 245-254.

Duan, J. J., and C. B. Oppel. 2012. Critical rearing parameters of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) as affected by host-plant substrate and host-parasitoid group structure. J. Econ. Entomol. 105: 792 - 801.

Duan, J.J., T. Watt, P. Taylor, K. Larson, and J.P. Lelito. 2013a. Effects of ambient temperature on egg and larval development of the invasive emerald ash borer (Coleoptera: Buprestidae): implications for laboratory rearing. J. Econ. Entomol. 106(5): 2101-2108.

Duan, J.J., L.S. Bauer, K.J. Abell, J.P. Lelito, and R. van Driesche. 2013b. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer. J. Econ. Entomol. 106: 1145-1154.

Duan, J.J., K. Larson, T. Watt, J. Gould, and J.P. Lelito. 2013c. Effects of host plant and larval density on intraspecific competition in the larvae of emerald ash borer (Coleoptera: Buprestidae). Eviron. Entomol. 42(6): 1193-1200.

Duan, J.J., T.J. Watt and K. Larson. 2014. Biology, life history and laboratory rearing of *Spathius galinae* (Hymenoptera: Braconidae), a larval parasitoid of

the invasive emerald ash borer (Coleoptera: Buprestidae). J. Econ. Entomol. 107(3): 939-946.

Drane, P., J. Sherwood., R. Colosimo, and D. Kretschmann. 2012. A study of wood baseball bat breakage. 9th conference of the international sports engineering association. Procedia Engineering 34:616-621.

Emerald Ash Borer Information. 2014. Emerald Ash Borer. [http://www.emeraldashborer.info/index.cfm].

Fellowes, M.D.E., J.J.M. van Alphen, and M.A. Jervis. 2005. Foraging behavior. p. 2-71. *In* Jervis, M.A. Insects as Natural Enemies: a Practical Perspective. Springer, Netherlands.

Force, D.C., and P.S. Messenger. 1964. Fecundity, reproductive rates, and the innate capacity for increase of three parasites of *Therioaphis maculata* (Buckton). Ecology. 45(4): 706-715.

Gandhi, J.K.J., and D.A. Herms. 2010. North American arthropods at risk due to widespread *Fraxinus* mortality caused by the alien emerald ash borer. Biol. Invasions. 12: 1839–46

Godfray, H.C.J. 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press. Princeton, NJ.

Gomez-Torres, M.L., D.E. Nava, and J.R.P. Parra. 2012. Life table of *Tamarixia radiata* (Hymenoptera: Eulophidae) on *Diaphorina citri* (Hemiptera: Psyllidae) at different temperatures. J. Econ. Entomol. 105(2): 338-343.

Gordh, G., E.F. Legner, and L.E. Caltagirone. 1999. Biology of parasitic hymenoptera. p. 355-381. *In* Bellows, T.S. and T.W. Fisher (eds.). Handbook of Biological Control. Academic Press, San Diego, CA.

Gould, J. R., T. Ayer, and I. Fraser. 2011. Effects of rearing conditions on reproduction of *Spathius agrili* (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer (Coleoptera: Buprestidae). J. Econ. Entomol. 104: 379-387.

Gould J. R., and J. J. Duan. **2013**. Petition for Release of an Exotic Parasitoid, *Spathius galinae* Belokobylskij & Strazanac, for the Biological Control of the Emerald Ash Borer, *Agrilus planipennis* Fairmaire. USDA APHIS. Petition 2013-01.

Haack, R. A., E. Jendek, H-P. Liu, K. R. Marchant, T. R. Petrice, T. M. Poland, and H. Ye. 2002. The emerald ash borer: a new exotic pest in North America. Newsletter of the Michigan Entomological Society 47(3-4): 1-5.

Hamilton, W.D. 1967. Extraordinary sex ratios. Science. 156: 477-488.

Harlow, W.M., E.S. Harrar, J.W. Hardin, and F.M. White. 1996. Textbook of dendrology, 8th ed. McGraw Hill, Inc. New York, NY.

Heinz, K.M. 1998. Host size-dependent sex allocation behavior in a parasitoid: implications for *Catolaccus grandis* (Hymenoptera: Pteromalidae) mass rearing programmes. B. Entomol. Res. 88: 37-45.

Herms, D.A. and D.G. McCullough. 2014. Emerald ash borer invasion of North America: history, ecology, impacts, and management. Annu. Rev. Entomol. 59: 13-30.

Hoelmer, K.A., and A.A. Kirk. 2005. Selecting arthropod biological control agents against arthropod pests: can the science be improved to decrease the risk of releasing ineffective agents? Biol. Control. 34, 255–264.

Infante, F. 2000. Development and population growth rates of *Prorops nastua* (Hym., Bethylidae) at constant temperatures. J. Appl. Ent.124: 343-348.

Irvin, N.A., and M. S. Hoddle. 2006. The effect of intraspecific competition on progeny sex ratio in *Gonatocerus* spp. for *Homalodisca coagulata* egg masses: economic implications for mass rearing and biological control. Biol. Control. 39: 162-170.

Iwasa, Y., Y. Suzuki, and H. Matsuda. 1984. Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. Theor. Popul. Biol. 26: 205-227.

Jervis, M.A., M.J.W. Copeland, and J.A. Harvey. 2005. The life cycle. p. 73-165. *In* Jervis, M.A. Insects as Natural Enemies: a Practical Perspective. Springer, Netherlands.

Ju, R.T., F. Wang, and B. Li. 2011. Effects of temperature on the development and population growth of the sycamore lace bug, *Corythucha ciliata.* J. Insect Sci.11:16 [available online: insectscience.org/11.16]

King, B.H. 1987. Offspring sex ratios in parasitoid wasps. Q. Rev. Biol. 62(4): 367-396.

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 US communities, 2009-2019. Ecol. Econ. 69: 569-578.

Lactin, D.J., N.J. Holliday, D.L. Johnson, and R. Craigen. 1995. Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24: 68–75.

Laughlin, R. 1965. Capacity for increase: a useful population statistic. J. Anim. Ecol. 34: 77-91.

Lelito, J.P., R. Lavellée, J.R. Lomeli, B. Lyons, and M. Marcotte. 2013. ST 03: Application of biological control of the emerald ash borer (EAB) in North America. NAPPO Science and Technology Documents.

Lopez, O.P., Y. Hénaut, J. Cancino, and M. Lambin. 2009. Is host size an indicator of quality in the mass-reared parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)? Fla. Entomol. 92(3): 441-449. doi: 10.1653/024.092.0305

Mack, R.N., D. Simberloff, W.M. Lonsdale, H. Evans, M. Clout, and F.A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecol. Appl. 10(3): 689-710.

MacFarlane, D.W. and S.P. Meyer. 2005. Characteristics and distribution of potential ash tree hosts for emerald ash borer. Forest Ecol. Manag. 213: 15-24.

Muirhead, J.R., B. Leung, C. van Overdijk, D.W. Kelly, K. Nandakumar, K.R. Marchant, and H.J. MacIsaac. 2006. Modeling local and long-distance

dispersal of invasive emerald ash borer *Agrilus planipennis* (Coleoptera) in North America. Divers. Distrib. 12: 71-79.

Nowak, D.J. 2010. Assessing urban forest effects and values: Chicago's urban forest. United States Dept. of Agr.-Forest Service-Northern Research Station. Resource Bulletin NRS-37.

Ode, P.J., and I.C.W. Hardy. 2008. Parasitoid sex ratios and biological control. *In* Wajnberg, E., C. Bernstein, and J. van Alphen (eds.), Behavioral ecology of insect parasitoids, from theoretical approaches to field applications. Blackwell publishing, Malden, MA.

Ode, P.J. and K.M Heinz. 2002. Host-size-dependent sex ratio theory and improving mass-reared parasitoid sex ratios. Biol. Control 24:31-41

Opp, S.B and R.F. Luck. 1986. Effects of host size on selected fitness components of *Aphytis melinus* and *A. lingnanensis* (Hymenoptera: Aphelinidae). Ann. Entomol. Soc. Am. 79: 700-704.

Parrella, M.P., K.M. Heinz, and L. Nunney. 1992. Biological control through augmentative releases of natural enemies: a strategy whose time has come. Am. Entomol. 38: 172-179.

Parry, D. 2009. Beyond Pandora's Box: quantitatively evaluating non-target effects of parasitoids in classical biological control. Biol. Invasions. 11: 47-58.

Patton, R.L. 1963. Introductory Insect Physiology. W.B. Saunders Co. Philadelphia, USA.

Pilkington, L.J., and M.S. Hoddle. 2006. Use of life table statistics and degree-day values to predict the invasion success of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an egg parasitoid of *Homalodisca coagulate* (Hemiptera: Cicadellidae), in California. Biol. Control 37: 276-283.

Pilkington, L.J., M. Lewis, D. Jeske, and M.S. Hoddle. 2014. Calculation and thematic mapping of demographic parameters for *Homalodisca vitripennis* (Hemiptera: Cicadellidae) in California. Ann. Entomol. Soc. Am. 107(2): 423-434. doi: http://dx.doi.org/10.1603/AN13144.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience. 50: 53-65.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated alien-invasive species in the United States. Ecol. Econ. 52: 273-288.

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.

Pratissoli, D., and J.R.P. Parra. 2000. Fertility life table of *Trichogramma pretiosum* (Hym., Trichogrammidae) in eggs of *Tuta absoluta* and *Phthorimaea operculella* (Lep., Gelechiidae) at different temperatures. J. Appl. Ent. 124: 339-342.

Prokopy, R.J., and A.H. Reynolds. 1998. Ovipositional enhancement through socially facilitated behavior in *Rhagoletis pomonella* flies. Entomol. Exp. Appl. 86: 281-286.

Prokopy, R.J., and D.B. Roitberg. 2001. Joining and avoidance behavior in nonsocial insects. Ann. Rev. Entomol. 46: 631-635.

Prokopy, R.J., and J.J. Duan. 1998. Socially facilitated egg laying behavior in Mediterranean fruit flies. Behav. Ecol. Sociobiology. 42(2): 117-122.

Qiu, B. Z-S. Zhou, S-P. Luo, and Z-F. Xu. 2012. Effect of temperature on development, survival, and fecundity of *Microplitis manila* (Hymenoptera: Braconidae). Envrion. Entomol. 41(3): 657-664.

Ramadan, M.M., T.T.Y. Wong, and D.O. McInnis. 1994. Reproductive biology of *Biosteres arisanus* (Sonan), an egg-larval parasitoid of the oriental fruit fly. Biol. Control. 4: 93-100.

Rosenheim, J.A. and D. Rosen. 1991. Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis*: distinguishing the effects of egg load and experience. J. Anim. Ecol. 60(3): 873-893.

Sala, O.E., *et al.* 2000. Global biodiversity scenarios for the year 2100. Science. 287: 1770-1774.

SAS Institute. 2012. JMP® Version 10.0.1. SAS Institute Inc., Cary, NC.

Scarr, T.A., D.G McCullough and G.M Howse. 2002. Forest Health Alert-*Agrilus planipennis* Emerald Ash Borer. Natural Resources Canada-Canadian Food Inspection Agency.

Siegert, N.W., D.G. McCullough, A.M. Liebhold, and F.W. Telewski. 2007. Resurrected from the ashes: a historical reconstruction of emerald ash borer dynamics through dendrochronological analyses. US Department of Agriculture Forest Service.

Simberloff D., and P. Stiling. 1996. How risky is biological control? Ecology. 77:1965–1974.

Simberloff, D. 2012. Risks of biological control for conservation purposes. BioControl. 57: 263-276.

Southwood, T.R.E., and P.A. Henderson. 2000. Ecological methods, 3rd ed. Blackwell Science, Oxford, UK.

Staubli Dreyer, B., P. Neuenschwander, B. Bouyjou, J. Baumgartner., and S. Dorn. 1997. The influence of temperature on the life table of *hyperaspis notata*. Entomol. Exp. Appl. 84: 85–92.

Taylor, F. 1981. Ecology and evolution of physiological time in insects. Am. Nat. 117(1): 1-23.

Ulyshen, M.D., J.J. Duan, and L.S. Bauer. 2010a. Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis*. 52: 188-193.

Ulyshen, M.D., J.J. Duan, L.S. Bauer, and I. Fraser. 2010b. Suitability and accessibility of immature *Agrilus planipennis* (Coleoptera: Buprestidae) stages

to *Tetrastichus planipennisi* (Hymenoptera: Eulophidae). J. Econ. Entomol. 103(4): 1080-1085.

Ulyshen, M.D., R.W. Mankin, Y. Chen, J.J. Duan, T.M. Poland, and L.S. Bauer. 2011. Role of emerald ash borer (Coleoptera: Buprestidae) larval vibrations in host quality assessment by *Tetrastichus planipennisi* (Hymenoptera: Eulophidae). J. Econ. Entomol. 104(1): 81-86. doi: 10.1603/EC10283.

U.S. Climate Data. 2014. [www.usclimatedata.com]

USDA–APHIS 2007. The proposed release of three parasitoids for the biological control of the emerald ash borer (*Agrilus planipennis*) in the continental United States: environmental assessment. Federal Register 72: 28947-28948. Docket APHIS-2007-0060.

Van den Bosch, R., P.S. Messenger, and A.P. Gutierrez. 1982. An introduction to biological control. Plenum Press, New York, NY.

Van Driesche, R.G., R.I. Carruthers, T. Center, M.S. Hoddle, J. Hough-Goldstein, L. Morin, L. Smith, D.L. Wagner, *et. al.* 2010. Classical biological control for the protection of natural ecosystems. Biol. Control. 54: S2-S33.

Waage, J.K. and S.M. Ng. 1984. The reproductive strategy of a parasitic wasp: I. optimal progeny and sex allocation in *Trichogramma evanescens.* J. Anim. Ecol. 53(2): 401-415.

Waage, J.K., K. Carl, N.J. Mills, and D.J. Greathead. 1985. Rearing entomophagous insects. *In* Singh, P. and R.F. Moore (eds.), Handbook of Insect Rearing. Elsevier Science Publishers, New York, NY,

Wang, X-Y., Z-Q. Yang, H. Hu, and J.R. Gould. 2008. Effects of host size on the sex ratio, clutch size, and size of adult *Spathius agrili*, an ectoparasitoid of emerald ash borer. Biol. Control. 44: 7-12.

Wang, X-Y., Z-Q. Yang, J.R. Gould, H. Wu, and J-H. Ma. 2010a. Host-seeking behavior and parasitism by *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer. Biol. Control. 52: 24-29.

Wang, X-Y., Z-Q. Yang, J.R. Gould, Y-N. Zhang, G-J. Liu, and E-S. Liu. 2010b. Biology and ecology of the emerald ash borer, *Agrilus planipennis,* in China. J. Insect Sci. 10(128): 1-23. [available online: insectscience.org/10.128]

Wang, X-Y., Y-L. Tang, J-F. Lu, and Z-Q. Yang. 2013. Biomass evaluation of concealed insect pests at parasitism based on allometric scaling laws. Phytoparasitica. 41: 435-441.

Werren, J.H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. Science. 208: 1157-1159.

Werren, J.H. 1983. Sex ratio evolution under local mate competition in a parasitic wasp. Evolution. 37(1): 116-124.

Westphal, M.I., M. Browne, K. MacKinnon, and I. Noble. 2008. The link between international trade and the distribution of alien invasive species. Biol. Invasions. 10: 391-398. doi: 10.1007/s10530-007-9138-5

Yang, S., J.J. Duan, T. Watt, K.J. Abell, and R.G. van Driesche. 2012. Responses of an idiobiont ectoparasitoid, *Spathius galinae* (Hymenoptera: Braconidae), to host larvae parasitized by the koinobiont endoparasitoid *Tetrastichus planipennisi* (Hymenoptera: Eulophidae): implications for biological control of emerald ash borer (Coleoptera: Buprestidae). Environ. Entomol. 41(4): 925-932. doi: http://dx.doi.org/10.1603/EN12072.

Yang, Z-Q., X-Y. Wang, J.R. Gould, R.C. Reardon, Y-N. Zhang, G-J. Liu, and E-S. Liu. 2010. Biology and behavior of *Spathius agrili*, a parasitoid of the emerald ash borer, *Agrilus planipennis*, in China. J. of Insect Sci. 10:30 available online: doi: 10.1673/031.010.3001