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*Non-native Pest*

# Biology and Control of Emerald Ash Borer



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# Chapter 8: Mass-rearing of Emerald Ash Borer and its Parasitoids

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## INTRODUCTION

Mass rearing of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) poses significant challenges both in terms of understanding its biology and phenology in the field and maintaining sources of insect material in the laboratory. Fortunately, in the past few years, significant progress has been made by several USDA facilities on optimizing laboratory rearing of emerald ash borer for a variety of purposes (Duan et al., 2013ab).

Rearing the natural enemies of wood boring insects is difficult because of the need for effective means to mass rear the host, which has a long (one or two year) life cycle and cryptic life history. At the initiation of any biological control program for a newly detected invasive wood boring pest such as EAB, information on the target pest as well as its natural enemies may be very limited. Using the emerald ash borer/parasitoid system as an example, the three primary natural enemies, now being produced and released across the United States, were newly described in the first several years following an intensive foreign exploration program (USDA APHIS, 2007) in response to the detection of the borer by federal agencies.

At their simplest, the critical resources required for mass production of natural enemies of emerald ash borer are growth chambers with good temperature, humidity, and photoperiod control, fresh ash foliage (preferably produced in the field), and small-diameter, clean ash logs from sapling trees (either from the field or grown in the greenhouse). An adequate supply of EAB larvae or adults can sometimes be harvested from the field for laboratory use from nearby EAB infestations, but laboratory rearing is recommended to minimize disease and maximize beetle fecundity. Below, we discuss the current best-practices for mass rearing the emerald ash borer and its parasitoids. It should be noted that as new parasitoids are introduced, their rearing may also be broadly similar to the methods presented here.

## MASS REARNG EMERALD ASH BORER

Rearing emerald ash borer in any life stage is time consuming. Foliage provided to adult beetles must be replaced at least every four days; an efficient system for doing so is to maintain two sub-colonies, in which insects are given fresh foliage either on Monday and Thursday or on Tuesday and Friday. This step requires providing the insects with fresh foliage in a clean water vial in a fresh, clean container. No additional water is necessary, although misting the foliage during the provisioning process preserves foliage health.

**Figure 1.** Sex determination of emerald ash borer beetles. Female is on left; note larger size and wider abdomen, especially the two segments just posterior to the hindmost pair of legs. Males also have a pronounced silvery pubescence (the "beard") on the ventral surface of the anterior thoracic segments. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

**Table 1.** Effect of number of females per cage on emerald ash borer fecundity and longevity.

| Number of Females at Initiation | % Enclosures w/Eggs | % Producing at 21 d | Mean Total Eggs | Mean Longevity (d) |
|---|---|---|---|---|
| 1 | 56.8 | 74.6 | 156 | 31 |
| 2 | 70.2 | 78.0 | 190 | 37 |
| 3 | 82.0 | 58.7 | 96 | 40 |

Optimal rearing of adult beetles, to maximize laboratory production of eggs, can be achieved using two females and two males (see Fig. 1 for sex determination) provisioned with foliage as soon as possible following adult eclosion, although a single female per cage is also useful for some experimental needs (Table 1). Higher numbers of insects per cage is not recommended. At least two males should be used, regardless of the number of females, to ensure mating success (Rutledge and Keena, 2012). Beetles are allowed to emerge from field-collected ash logs in large cardboard barrels with funnels and jars at one end, and the beetles are collected in jars, to which beetles are drawn by light (Fig. 2). Alternatively, beetles can be reared through their entire life cycle in the laboratory in small ash logs (see below). Ash leaves used to feed adult beetles must be clean and free of pest damage (any decrease in nutrient content or increase in plant defense compounds will reduce EAB fecundity). The species of ash used as the source of foliage has significant effects on fecundity, with field-collected foliage of mature green ash (*Fraxinus pennsylvanica* Marshall) being the best for mass-rearing. During the winter, dark green, mature foliage from greenhouse-grown tropical ash (*Fraxinus uhdei* [Wenz.] Lingelsh.) can be substituted for green ash, but is generally inferior in quality to green ash and results in lower beetle fecundity. The EAB Rearing Facility in Brighton, Michigan (USA) has overcome this problem by having fresh young *F. uhdei* foliage shipped from southern California, where it grows as an ornamental. While this source of foliage is a more suitable than that from greenhouse-grown trees, shipping is expensive when considered over the course of a year.

To house the adult beetle colony, the EAB Rearing Facility uses 946 mL clear plastic cups and ventilated mesh lids (Fig. 3) such as those available from the following source: http://www.joshsfrogs.com/32-oz-insect-cup-and-lid-placon-cup-250-pack.html.

**Figure 2.** Cardboard rearing barrels, with funnels and vials inserted into the lids, into which emerald ash borer adults collect following their emergence from logs inside barrels. This is an efficient system for rearing adult beetles from field-harvested beetle-infested logs. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

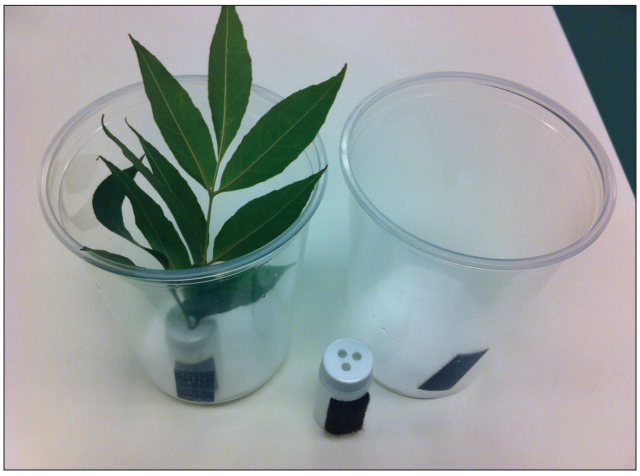

**Figure 3.** Emerald ash borer enclosure and setup showing 946 mL plastic cup, Velcro, water vial with drilled lid (right), and ash foliage added (left). This is an appropriate amount of ash foliage for 3-4 insects for 3 days. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

**Figure 4.** Emerald ash borer rearing enclosures showing screen and filter paper lids secured for oviposition. Tracking egg production data is useful for optimizing colony demography. A simple data sheet, visible on side of cup, records the day of beetle emergence, weekly egg production, and the number of beetles dead at each provisioning with new foliage. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

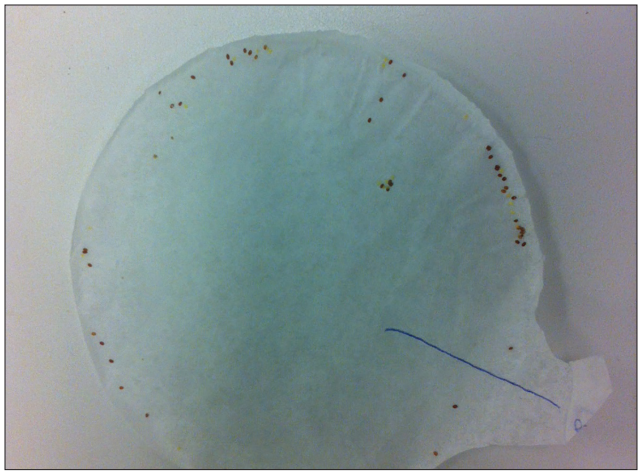

**Figure 5.** Filter paper removed from the top of an emerald ash borer-rearing enclosure, showing eggs (brown dots). Number of eggs shown is typical production from two emerald ash borer females for 3-4 days. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Inside this plastic cup, a small 5-6 cm diameter disk of paper towel is placed on the bottom to absorb moisture from frass and foliage. A 3-4 cm strip of Velcro (Sticky Back Velcro Tape, 8 m x 1.9 cm, www.uline.com) is applied to the inner surface of the plastic cup. The matching portion of Velcro is fixed to the cap of a 20 mL plastic scintillation vial (Fisher Scientific, Product # 03-341-72A). Several 5-7 mm diameter holes are drilled into the vial's cap. The vial thus serves as a removable water reservoir to keep ash foliage alive while the beetles feed (Fig. 3). Vials, fiberglass screens, ventilated mesh lids, and plastic cups are bleached after each use. Paper toweling and filter paper lids are replaced each time foliage is changed. EAB-rearing cages are kept in a walk-in growth chamber held at 27 (± 1° C) during the day and 22 (± 1° C) at night, with a 16:8 light-dark cycle. Relative humidity is held at 75-80 (± 5%). Groups of 3-4 EAB adults are housed this way for two weeks, at which time the type of lid used is changed to facilitate egg-laying and collection. The cage construction remains the same, but instead of a ventilated lid, a 10 x 10 cm square of black fiberglass window screen is placed on top of the plastic cup. On top of this, a single coffee filter paper (Meijer Brand, 8-12 cup size) is placed, and both are tightly secured against the plastic cup with several small rubber bands. EAB adults perceive the fiberglass screening as a rough surface under which to deposit eggs (Fig. 4); eggs thus laid adhere to the easily removable filter paper, which can then be used either to rear egg parasitoids or EAB larvae (Fig. 5).

Egg production and EAB health should be closely monitored to ensure rearing success (data sheets on the front of cages assist in this effort; Fig. 4). Any insects that die should be promptly removed from their cages, and any cage that loses more than two of the initial four insects should be discarded. Any evidence of fungal infection, such as sporulating cadavers, must be dealt with swiftly, as outbreaks can quickly devastate a large colony. The best methods to limit infection and outbreaks are to thoroughly clean all supplies, inspect rearing cages before opening to prevent transfer of pathogens (e.g., "infected" cages are discarded unopened), and remove dead insects promptly. Any beetles showing reduced fecundity should immediately be isolated. Any rearing cage that fails to produce eggs by day 21 post-adult EAB eclosion should be discarded even if the beetles remain alive – it is likely these insects are of poor quality or diseased, as the majority of healthy EAB females will lay eggs by this time (Table 1). Egg production naturally declines after 9-10 weeks of adult life, and to maintain efficiency and limit disease

**Figure 6.** An ash log artificially infested with emerald ash borer eggs. Small pieces of filter paper bearing beetle eggs are secured to the logs, eggs facing inward to facilitate establishment of young larvae. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

**Figure 7.** Emerald ash borer larvae can be reared in freshly cut green ash logs (14 cm tall by 5-8 cm wide) held in plastic trays (25 x 30 cm) filled with 1-2 cm of clean water. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

all rearing groups should be discarded after this age or as soon as egg production begins to decline.

EAB eggs deposited on coffee filters (Fig. 5) provide a convenient means to transfer eggs to ash logs, for the production of EAB larvae and pupae. EAB eggs can be gently secured to ash logs (Fig. 6) using a strip of Parafilm (Fisher Scientific, Cat. No. S37440), and inoculated logs then allowed to develop in trays of clean water for several weeks (Fig. 7). The EAB Rearing Facility uses photographic developing trays that are available commercially (Cescolite, Item # CL1114T) to maintain logs at this stage. Trays of this type are advantageous because excess water can be easily poured out, and trays hold a large number of ash logs and are highly resistant to chemicals, allowing trays to be easily sterilized with bleach after use. Larvae serve as hosts for the larval parasitoids (see below) or can be allowed to mature and excavate pre-pupal cells, after which time they can be used for the production of EAB adults. Temperature has a significant effect on EAB development and rearing temperatures should generally be at or below 30°C for optimal development (Duan et al., 2013a), especially for adult beetles. A key exception may be the rearing EAB larvae at slightly higher temperatures (i.e., 32-33° C) for more rapid production of larvae to serve as hosts for the larval parasitoids (see below). At this temperature, mature, 4$^{th}$ instar larvae can be produced in about three weeks.

Production of EAB adults using the method described above for rearing larvae is advantageous, although time consuming. Where wild-collected material is available, trees infested with numerous EAB can be felled and later warmed for beetle emergence in the laboratory. Field-collected material is only available seasonally, however, and long-term storage (e.g., from winter of the current year until the next autumn or winter) severely decreases the quality of the insects produced. To avoid these problems, EAB-infested ash logs can be incubated at warm temperatures for several months to allow full larval development, then chilled for several months, and later warmed for the production of EAB adults. Laboratory-reared adults suffer lower incidence of disease and generally have much higher fecundity than field-collected EAB adults because the duration of cold storage can be precisely controlled.

## MASS REARING EAB PARASITOIDS

### *Oobius agrili*

*Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), a solitary egg parasitoid of EAB, can easily be reared with some modifications of the methodology developed by Liu and Bauer (2007). All laboratory colonies are parthenogenetic, and each female is capable of successfully parasitizing

at least several dozen EAB eggs in her lifetime (Liu and Bauer, 2007). An efficient mass-rearing system has been developed using a two-generation system: post-diapause *O. agrili* rearing cages are kept in a walk-in growth chamber at 25 (± 1° C), with a 16:8 light-dark cycle. When provisioned with EAB eggs, these insects will produce progeny, a majority of which emerge within three to four weeks. Some diapausing progeny will be produced under long-day conditions (generally around 20% of the total) and can be separated by examination under a microscope after non-diapausing progeny are allowed to emerge, by collecting those parasitized eggs that have no exit hole. It is important to note that this process is extremely time-consuming in a mass-rearing setting, where more than ten thousand EAB eggs must be examined per week, and is recommended only if all progeny must be collected for specific experimental needs. Non-post-diapause *O. agrili*, produced as outlined above, are transferred on the day of eclosion to rearing cages kept in a walk-in growth chamber held at 25 (±1° C), with an 8:16 light-dark cycle. Relative humidity under both photoperiod regimes is held at a constant 75-80 (± 5%). The non-post-diapause individuals, reared under short-day conditions, will produce diapausing progeny, which can then be stored for up to ten months at 4 ± 1° C and high (>75-80%) relative humidity. Storing *O. agrili* in diapause can be done as follows: 21 days after first exposure of fresh EAB eggs to adult *O. agrili*, the parasitized eggs are transferred into clean cups (i.e., no honey) and moved from 25° C to 10° C. After one week at 10° C, parasitized eggs are transferred to 4° C until needed. Post-diapause adults begin to emerge from cold-stored material after about one month of being returned to 25° C, and the cycle can be repeated.

*Oobius agrili* adults are very small and can easily crawl through very small openings, including all types of screen tested thus far (even insect netting), so care must be taken to maintain proper housing or adults will readily escape. *Oobius agrili* wasps can be securely housed in clear plastic 473 mL cups (Gordon Food Service, Item # 7922500) fitted with very tight, solid clear-plastic lids (Solo Brand, Item # 626TP-0090). Lids can be re-used, but must be assessed for tight fit – if the lid is used many times, it can become deformed and this may allow insects to escape. Nutrition must be provided to the wasps to ensure a normal life span of 2-3 weeks and optimal progeny production. Honey can be streaked directly onto the interior walls of the plastic cup using a very fine tool, such as a single hair from a brush. Care must be taken to ensure that the streaks are fine (<0.25 mm) so that the wasps do not become trapped as they attempt to feed. The relative humidity in the rearing environment will cause the honey streaks to absorb moisture, and this will provide *O. agrili* adults with sufficient water. No more than 5-6 streaks are needed per enclosure for a 1-wk period.

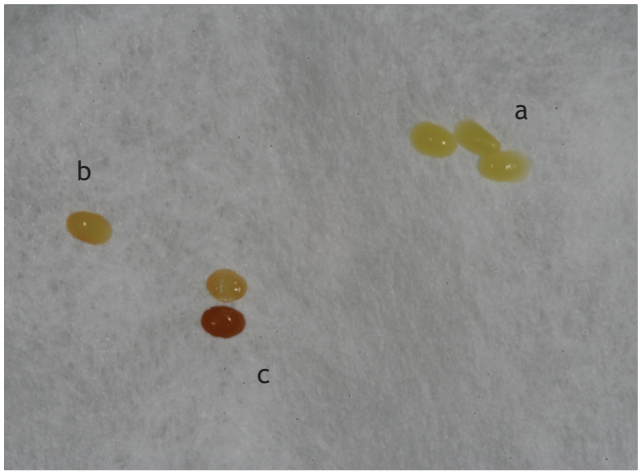


**Figure 8.** Emerald ash borer eggs at various stages of development. (a) freshly laid, suitable for oviposition of *Oobius agrili;* (b) 24-36 hours old, suitable for oviposition by *Oobius agrili;* (c) upper egg deflated and perhaps damaged; lower egg 2-3 days old and less likely to be parasitized unless presented immediately. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Healthy EAB eggs, deposited on filter papers (Fig. 5), can be provided to *O. agrili* females beginning on the day of their emergence. Groups of up to 20 females can be held together in a single 16 oz. plastic cup, stocked with fresh EAB eggs once per week at a rate of 3-5 EAB eggs per *O. agrili* female. This generally results in parasitism rates of greater than 75%. Lower rates commonly result from using older EAB eggs (>3-4 days post-harvest), many of which will develop to near hatching during the course of exposure to *O. agrili* adults and will not be parasitized (Fig. 8). Groups of *O. agrili* adults in which no significant mortality has occurred, can be re-used for an additional week by moving them to a new,

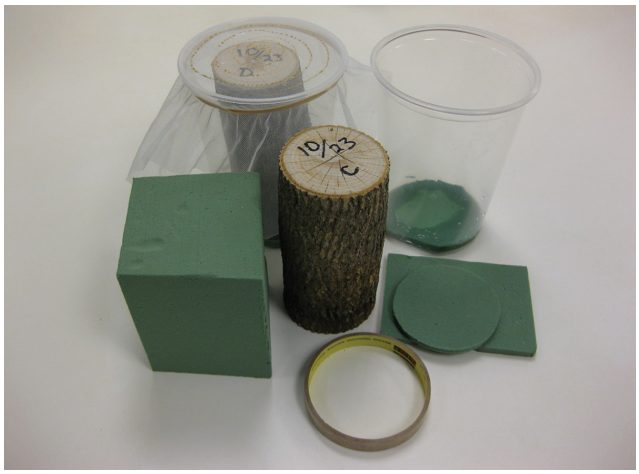

**Figure 9.** Supplies needed for creation of larval-bolt exposures for rearing emerald ash borer larval parasitoids. Clockwise from upper left: completed exposure cage with honey, empty cup with floral foam disk, sliced rectangle and circle cut with masking tape roll, floral foam brick and EAB-infested ash log (center). (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

**Figure 10.** Groups of *Spathius agrili* females are more efficient at parasitism and progeny production than single individuals. Several females often aggregate over unparasitized hosts; after parasitism occurs by one or more (often several) individuals, the group disperses and reforms over the gallery of another host. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

clean cup freshly streaked with honey, to which the appropriate number of EAB eggs have been added. Transferring *O. agrili* adults between cups is best accomplished by removing all other material from the cup (filter papers, dead wasps, and hatched EAB larvae), and then simply tapping the live wasps into the new cup. Fecundity decreases rapidly in groups of females more than two weeks of age and re-using adults for a third exposure is not recommended.

### *Spathius agrili*

*Spathius agrili* Yang (Hymenoptera: Braconidae) is a gregarious idiobiont larval ectoparasitoid of EAB. Adult females are robust, capable of living for several months, and able to produce several dozen progeny during this time (Gould et al., 2011). At 25-27° C, one month is required from adult emergence to the production of new adults. New adult females need to be separated into groups of not more than 20-25 soon after emergence to prevent mortality from crowding. *Spathius agrili* can be easily housed in the same 473 mL cups as *O. agrili*, with the modification of using insect netting (www.skeeta.com, 625 holes per sq. in.) secured over the opening of the cup with a rubber band, rather than a solid lid. Honey is streaked into this screen (and replaced as it is consumed) and wasps are misted daily with clean, reverse osmosis water.

To mass rear *S. agrili* (and *Tetrastichus planipennisi* Yang as well, see below), EAB larvae are reared in small-diameter ash bolts in the laboratory until they reach the appropriate stage, and then host-infested logs are exposed to groups of adult wasps. Using a simple set of supplies (Fig. 9), EAB-infested ash logs can be exposed to parasitoids. The same 946 mL cups used to hold adult EAB beetles can be used here too, with minor modifications. Instead of a pad of paper towels, a disk of floral foam is added to the bottom of the cup to retain moisture. Floral foam bricks (http://www.save-on-crafts.com/artesia.html) are sliced into thin (4-6 mm) rectangular sections using a sharp knife. The inner cardboard circle from a roll of masking tape can be used as a circular "knife" to cut disks of floral foam from these rectangular slices; disks are then placed in the bottom of the plastic cups. The EAB-infested ash log is then firmly pushed into the floral foam, and clean water added until the foam is saturated. Wasps are added using an electric aspirator, a piece of insect netting is secured over the opening of the cup with a rubber band, and the netting is streaked with honey to provide nutrition to the adult wasps (Fig. 9). The setup is then held at 25-27° C and 75-80% relative humidity, under a 16:8 light-dark cycle, for one week. After this incubation period, the adult wasps can be removed with an aspirator and re-used in another exposure. The parasitoid-exposed log is then incubated under

the same conditions, and adults emerge in 2-3 weeks.

Using the methods outlined above (see section on EAB) to produce ash bolts bearing EAB larvae, logs set up to rear EAB should be exposed to parasitoids between days 24 and 26 post-setup, to ensure that the larval hosts have achieved the greatest possible mass, and yet have not burrowed into the xylem, where they are inaccessible to the parasitoids. Each rearing cage should be stocked with 10 *S. agrili* females and 2-3 males and an EAB-infested ash log. This group of wasps can be used for up to three additional 1-wk exposures to fresh EAB-infested ash logs. Groups of females often form oviposition aggregations and a group size of 8-10 is best to facilitate rapid parasitism of most hosts in a container (Fig. 10). Groups with moderate mortality (2-3 dead females) during their second and third exposures to hosts can generally be re-used, combining wasps as necessary to keep group size in the optimal range, provided no signs of disease, such as sporulating cadavers, are present. If any evidence of disease is detected, all wasps in the affected group should be discarded.

To store *S. agrili* during the winter, diapause can be induced during the wasp's larval stage by manipulating temperature and photoperiod (Belill and Lelito, 2011). However, emergence from diapause occurs over several months and is relatively unpredictable. Holding some wasps in diapause (at immature stages) is, therefore, not particularly useful for mass-rearing since production cannot be well enough timed to produce synchronized groups of parasitoids for release. The method does have some value for storing field-collected material for long periods and for limiting the number of generations that a colony is subjected to laboratory rearing. Adults from cocoons (containing mature larvae) stored under cold conditions for several months have lower fecundity and higher mortality (Gould et al., 2011). Methods for storing *S. agrili* that bypass the need for diapause are still under development.

### *Spathius galinae*

*Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) is a recently described EAB parasitoid from the Russian Far East and South Korea (Belokobylskij et al., 2012). It was imported to the USDA ARS Beneficial Insects Quarantine Facility (Newark, Delaware) in 2010 and its host range has been studied to estimate the safety of its release in the United States against the emerald ash borer (Gould and Duan, 2013). A petition for field release of *S. galinae* was submitted to the USDA APHIS and NAPPO in March of 2013 for regulatory review and approval. While not yet (January, 2015) approved for field-release, a positive response from NAPPO and USDA APHIS has been issued to the petition's scientists and the parasitoid's potential future mass-rearing is described here. Based on its distribution in the Russian Far East and other part of northeast Asia, climatic matching suggests that it is more suitable for introduction against emerald ash borers in the northeast United States and Canada (Duan et al., 2012; Gould and Duan, 2013) than the previously introduced Chinese parasitoids (e.g., Liu and Bauer, 2007; USDA APHIS, 2007).

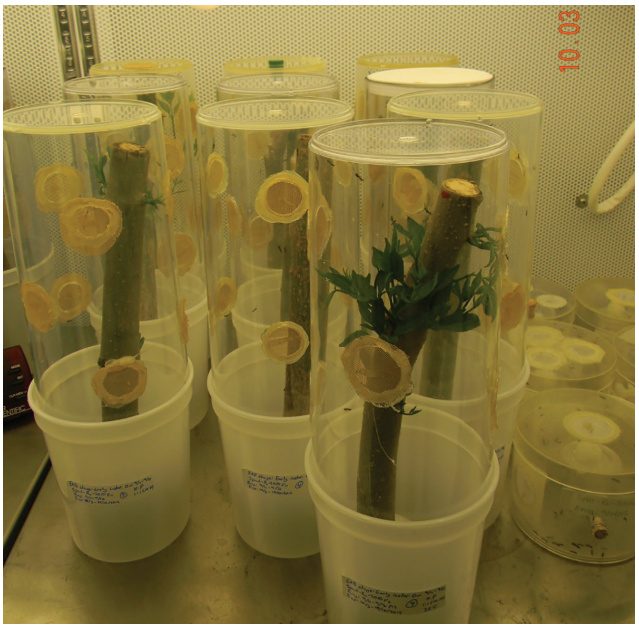


**Figure 11.** Exposure arena for production of *Spathius galinae*, containing five gravid female parasitoids and five males, as well as tropical ash logs infested with late instars of emerald ash borer. (Photo by Timothy Watt and Jian Duan (USDA ARS BIIR))

Specific rearing methods have recently been developed at the USDA ARS Beneficial Insects Research Unit. The first step is the exposure of mated female wasps to 3rd-4th instars EAB larvae naturally reared on freshly cut green or tropical ash logs (Fig. 11). *Spathius galinae* takes about one month (29 d)

to complete a generation (from egg to adult) under laboratory conditions (25 ± 1° C, 65 ±10% RH, L:D 16:8 h photoperiod). During this period, *S. galinae* larvae molt four times to reach the 5th instar, which then spins a cocoon for pupation and development to the adult stage. Adult female wasps survive seven weeks on average, with the peak oviposition occurring after three weeks when wasps are reared in groups, or after two weeks when wasps are reared as single pairs. Throughout its lifespan, one *S. galinae* female produces an average of 31 progeny (range 12-41) when reared in groups, but many more offspring (ave. 47, range 5-94) when reared as single pairs. Thus, in mass rearing *S. galinae*, adult wasps can be exposed to hosts for several weeks. Although *S. galinae* can be reared in emerald ash borer larvae in either green or tropical ash sticks, the rate of non-emergence of *S. galinae* progeny was much higher (20%) when wasps were reared on hosts in green ash sticks than in tropical ash sticks (2.1%).

Temperatures below 15° C induce mature *S. galinae* larvae (inside cocoons) to enter an obligatory diapause. Once in diapause, a minimum of 1-3 months of chill at 3-12° C is required to break diapause and permit development to the adult stage. Specific mechanisms that induce and break diapause for this species are still being investigated. Unlike *S. agrili*, we have found that material can be stored up to six months with no decrease in adult emergence; however, it is not known if cold storage affects fitness or performance of adults.

### *Tetrastichus planipennisi*

*Tetrastichus planipennisi* (Hymenoptera: Eulophidae) is a gregarious koinobiont larval endoparasitoid of EAB. The rearing method for *T. planipennisi* is similar to that for *S. agrili*, with a few key differences. First, rearing logs used should be from smaller trees whose bark is not more than 3 mm thick (Ulyshen et al., 2010; Abell et al., 2012). Second, the number of parasitoid adults per exposure cage should be slightly larger (12-15) than that used for *S. agrili*. Third, ash logs used to rear EAB larvae should be exposed to *T. planipennisi* a few days earlier (22-24 days post-setup of the EAB rearing) than for *S. agrili* to ensure

**Figure 12.** When nearly mature, the larvae of *Tetrastichus planipennisi* confer a braided appearance on their host larva. At this point or just afterwards, *T. planipennisi* broods can be induced into a state of torpor and be stored at low temperatures for up to six months without significant mortality. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

that the wasps have time to locate and parasitize all available EAB. Finally, groups of *T. planipennisi* females should only be used twice, as further use results in fewer progeny. Rearing of *T. planipennisi* is optimal at 25° C, 75-80% relative humidity, and a 16:8 light-dark cycle. Honey should be available to insects at all times, streaked on lids of rearing containers.

Another key difference between *T. planipennisi* and *S. agrili* is that *T. planipennisi* can be induced into a torpor state late in larval development, and in this condition *T. planipennisi* can be stored for long periods at 4 (±1° C) provided relative humidity is >75-80%. To induce torpor, immature parasitoids are chilled to 10° C on day 14 after host exposure to adult wasps, which is approximately when *T. planipennisi* larvae break out of their host larvae (Fig. 12). Logs can be held on moist floral foam in the rearing cages or they can be transferred into trays of shallow, clean water. Seven days after being placed at 10° C, rearing logs are transferred (remaining on moist foam or in shallow, clean water) to 4° C for storage. Mortality is generally <10% of the total cohort in each log when stored for up to six months. Under these conditions, most deaths are caused by bark drying and contracting, which crushes or traps some insects. Independent of bark desiccation, mortality increases during storage, as insects deplete metabolic reserves. Following return of stored wasps to 25° C,

*T. planipennisi* adults will usually emerge within 14 days, although some emergence may occur through day 21. Thus, unlike diapausing *S. agrili*, larvae of *T. planipennisi* can be stored in a manner that allows emergence of wasps to be predicted and controlled.

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