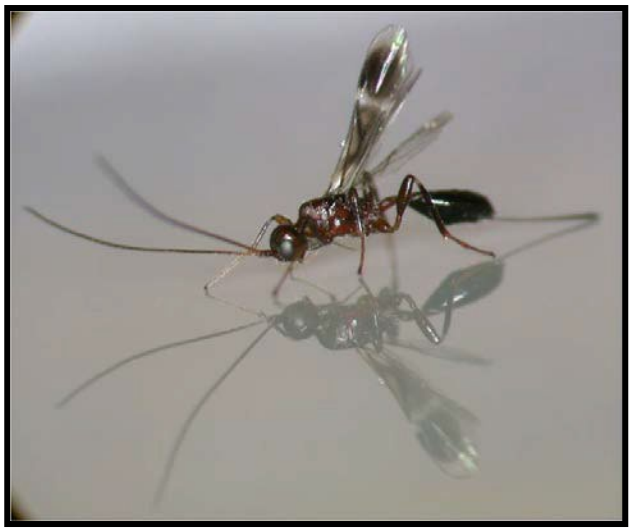
Emerald Ash Borer Biological Control Release and Recovery Guidelines 2024



United States Department of Agriculture
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US Forest Service
Cooperating State Departments of Agriculture

Emerald Ash Borer, *Agrilus planipennis* (Fairmaire), Biological Control Release and Recovery Guidelines 2024

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Cover photographs by: Top right: David Cappaert, Contractor, Michigan State University (*T. planipennisi*); Middle left: Tracy Ayer, Technician, USDA APHIS PPQ CPHST (*S. agrili*); Bottom right: Deborah Miller, Entomologist, USDA FS NRS (*O. agrili*)

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Contents

We also offer a comprehensive series of three pre-recorded video training sessions. The video sessions review the necessary tools and resources to choose an EAB parasitoid release site, conduct releases and perform recoveries. The videos will enhance your understanding of the guidelines and the science used to develop them. To access the training videos, visit the USDA APHIS EAB webpage (USDA APHIS | Emerald Ash Borer). The videos are listed under **Related Resources > Information for Cooperators > Training Videos.** Links to the videos are provided below for your convenience.

Module 1 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 7, 2022 - YouTube
An introduction and background and lifecycle of EAB, how to identify EAB and possible look-a-likes, history of biological control of EAB, how to identify ash and possible look-a-likes, impacts of EAB parasitoids and quiz on tree species and insect id. (1 hour 40 minutes)

Module 2 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 14, 2022 - YouTube
Mass rearing of EAB biological control agents for release, choosing sites and considerations for releasing parasitoids, conducting parasitoid releases, and an introduction to MapBiocontrol. (1 hour 47 minutes)

Module 3 Emerald Ash Borer Release and Recovery Guidelines – April 21, 2022 - YouTube
Evaluating parasitoid establishment: debarking for *oobius agrili*, evaluating parasitoid establishment: peeling trees for larval parasitoids, a tree peeling quiz and evaluating parasitoid establishment: yellow pan traps and yellow pan trap ID quiz. (2 hours 21 minutes)

INTRODUCTION AND BRIEF HISTORY OF EAB IN NORTH AMERICA

Emerald ash borer (EAB), a beetle from Asia that feeds on ash trees, was discovered as the cause of extensive ash mortality in southeast Michigan and adjacent areas of Canada in 2002. It is thought that this destructive pest was introduced in the early 1990's in infested solid wood packaging material originating in Asia. EAB feeds on ash species in the genus *Fraxinus* causing widespread ash mortality and significant economic, environmental, and social impacts.

Shortly after EAB was discovered in North America, federal and state regulatory agencies placed infested counties under quarantine and eradication activities were initiated. Due to the magnitude of the EAB infestation in North America, the potential for natural and artificial dispersal of EAB, limited EAB detection and control methods, and high costs, program objectives shifted away from eradication to containment and management of the pest.

As of March 2024, EAB infestations in the U.S. were known in Alabama, Arkansas, Colorado, Connecticut, Delaware, District of Columbia, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, and

Wisconsin and the Canadian provinces of Manitoba, New Brunswick, Nova Scotia, Ontario, and Québec.

The continued spread of EAB threatens our ash resources and will permanently alter forest ecosystems in North America. Ash wood is used for a variety of applications including tool handles, baseball bats, furniture, cabinetry, solid wood packing materials, pulp, and paper. Native Americans utilize black ash for basketry, which is both economically and culturally important. In addition to its value to the timber industry and the forest ecosystem, ash was a popular landscape tree and the spread of EAB has urban, suburban, and nursey industry impacts as well. The program aims to maintain ash as a viable part of the American landscape by managing known infestations with a robust biological control program, while also incorporating other IPM methods and outreach to minimize EAB's impacts.

LIFECYCLE OF EAB

EAB takes one or two years to complete its lifecycle depending on temperature, latitude, altitude, local population density, and tree health. Below is a description of the EAB lifecycle:

Adults

EAB adults begin to emerge from ash trees after the accumulation of 400-500 growing degree days base 50°F (GDD50F). Peak adult activity occurs at ~1,000 GDD. After emergence, adults fly into the ash canopy where they feed on leaves throughout their lives. EAB adults start mating one week after emergence, and females begin laying eggs 2-3 weeks later. In the field, EAB adults are readily observed mating and egg-laying on ash trees on warm, sunny afternoons. The adults of both sexes are strong fliers.

Eggs

A female EAB may lay >200 eggs in her lifetime, depositing them individually or in groups on the bark along the trunk and portions of major branches. Eggs are laid in areas where the bark is rough, and between bark layers or in bark crevices. Eggs are approximately 1.0 mm long x 0.6 mm wide and creamy white when laid; fertile eggs gradually turn amber after a few days (Appendix A). The eggs hatch after two to three weeks.

Larvae

Newly hatched larvae bore through the bark to the cambial region where they feed until mature. There are four stages (instars) of larval development (Appendix A). As they feed, the larvae create serpentine galleries filled with frass (excrement), which enlarge in width as the larvae grow (Appendix A). Larvae are creamy white, and dorso-ventrally flattened (Appendix A). When fully mature, 4^{th}-instar larvae are 26 to 32 mm long. The head is mostly retracted into the prothorax with only the dark brown mouthparts

visible. The prothorax is enlarged, with the mesothorax and metathorax slightly narrower. Larvae have 10 bell-shaped abdominal segments and a posterior pair of small brown structures called urogomphi (Appendix A).

Overwintering larvae, prepupae, pupae, and adults

In the fall, mature 4th-instar EAB larvae excavate pupal chambers in the new sapwood or outer bark where they fold into overwintering "J-shaped larvae" (Appendix A). In winter and spring, the J-shaped larvae shorten into prepupae then shed their cuticle to become naked pupae. Pupae are initially creamy white, but the eyes turn red, and the body darkens as they develop to the adult stage (Appendix A). To emerge from ash trees, adults chew D-shaped exit holes (Appendix A) through the bark and are immediately capable of flight upon emergence. EAB larvae that are immature as cold weather arrives in the fall will simply overwinter in their larval feeding gallery. While "J-shaped larvae" will pupate and complete development (i.e. become an adult beetle) the next spring, younger larvae may require another summer of feeding before pupating and becoming adult beetles the following spring (taking two years to develop).

DAMAGE AND SIGNS OF INFESTATION

EAB larvae damage ash trees by feeding on the phloem and cambium. In a new infestation, when just a few EAB larvae infest a tree, the tree responds by forming scar tissue or "callus" around EAB galleries, and the tree may show few outward signs of infestation. On some trees or branches, however, the callus may cause the bark to split open, exposing the EAB gallery beneath (Appendix A). Although difficult to detect, especially high in the canopy, the D-shaped exit holes chewed by emerging adults are diagnostic indicators of EAB infestation (Appendix A).

As EAB larval population density increases, the movement of nutrients through the phloem is disrupted and evidence of tree stress increases such as yellow foliage on dying branches, dead branches, small leaves, thinning crowns, and epicormic shoots (Appendix A). Woodpeckers feed on EAB larvae living under the bark of trees. Field observations suggest woodpecker feeding is one of the best indicators of early EAB infestation with the most obvious symptoms including bark scaling (removal of bark flakes) or 'blonding' due to the exposed bark being lighter in color and feeding holes through the bark (Appendix A).

HOST RANGE OF EAB

In North America, EAB attacks ash species in the genus *Fraxinus*, including but not limited to green ash (*F. pennsylvanica*), white ash (*F. americana*), black ash (*F. nigra*), pumpkin ash, (*F. profunda*), blue ash (*F. quadrangulata*), and Oregon ash (*F. latifolia*). Sixteen native species of ash, some with limited distributions in North America, are now threatened by EAB. In China, native ash species,

including Chinese ash (*F. chinensis*) and Manchurian ash (*F. mandshurica*), are less susceptible to EAB than North American species commonly planted in China such as velvet ash (*F. velutina*) and green ash (*F. pennsylvanica*). In 2014, EAB was observed attacking North American white fringetree, *Chionanthus virginicus L.* in Dayton, Ohio. Cipollini et al. 2017 found that EAB can attack and develop on cultivated olive, *Olea europaea L.*, However, the impact of EAB on white fringetree and cultivated olive is not yet well understood.

BIOLOGICAL CONTROL (BIOCONTROL) OF EMERALD ASH BORER

Biological control is the practice of importing and releasing natural enemies from a pest's native range to control the target pest populations. Biocontrol has been used for over 100 years in the U.S. It has successfully controlled invasive plant and insect pests such as spongy moth, winter moth, ash whitefly, eucalyptus longhorned borer, purple loosestrife, and Klamath weed. Because EAB is from northeast Asia, U.S., Chinese, and Russian scientists have been searching for EAB and its natural enemies in that region since 2003. In Asia, EAB population densities are relatively low. This is because of EAB-resistance in Asian ash species, ash scarcity and patchiness of forests, and the natural enemy complex. Finding EAB natural enemies in China, Russia, and Korea has yielded several hymenopteran parasitoids. Four species are approved for release as biological control agents of EAB in the U.S. Other species are under consideration.

BIOLOGY OF EAB BIOCONTROL AGENTS

Oobius agrili parasitizes up to 60% of EAB eggs laid during the summer in some areas of China. Tiny female *Oobius* accomplish this by searching the bark of ash trees for EAB eggs. When *Oobius* finds an EAB egg, it injects its own egg inside (Appendix B) where it will hatch, grow, and kill the host egg. All *Oobius* being released are females that reproduce without mating to produce only daughters. *Oobius* adults will emerge and repeat the cycle for at least two generations during the EAB egg-laying season. Each *Oobius* adult parasitizes up to ~80 EAB eggs during its lifetime. *Oobius* spend the winter as larvae inside EAB eggs and emerge as adults the following spring.

Spathius agrili parasitizes up to 90% of EAB larvae in ash trees east of Beijing in Tianjin, China, where the climate is relatively mild. So, releases of *S. agrili* are limited to EAB infestations in the south, where EAB also has a one-year life cycle like that of EAB in Tianjin, China. *Spathius agrili* is now released in areas where at least 50% of the EAB have a one-year life cycle. Modelling predicts this will be in areas that accumulate more than 3,500 GDD 50F (Appendix F). Female *Spathius* parasitize EAB larvae by drilling through the bark (Appendix B) and laying an average of 8 eggs on the outside of its host while simultaneously paralyzing the EAB. *Spathius agrili* is an ectoparastoid that lives externally on EAB. The hatching parasitoid larvae (Appendix B) feed and develop on the EAB larva, causing its death. The cycle is repeated 1-2 times each summer and fall depending on climate.

Spathius agrili overwinter as larvae or pupae and enter obligate diapause in the host gallery. Mature larvae spin silken cocoons in which they pupate and emerge as adults during the following summer.

Tetrastichus planipennisi is another larval parasitoid of EAB collected from China. The life cycle of *Tetrastichus* is similar to that of *Spathius,* however, the female parasitoid lays eggs inside EAB larvae where the parasitoid larvae grow, eventually killing their host. *Tetrastichus planipennisi* is an endoparasitoid that lives internally inside EAB, emerging after it kills and eats its host. *Tetrastichus* completes several generations each year, and one EAB larva can produce up to 130 *Tetrastichus* adults. They survive the winter under the bark of ash trees as larvae inside their host or as prepupae in their host gallery (Appendix B). As the weather warms in spring, the overwintering larvae of *Tetrastichus* gradually pupate, develop into adults, emerge from small round exit holes chewed in the bark above the gallery, and seek EAB larvae to parasitize. Due to the short ovipositor of *Tetrastichus*, they are more successful in parasitizing EAB larvae in small diameter ash sapling and trees up to ~6 inches in diameter at breast height (DBH). Research has shown that for *Tetrastichus* populations to persist they need EAB larvae to parasitize when they emerge in the spring. *Tetrastichus* is now preferentially released in areas where at least 25% of the EAB have a two-year life cycle and where modelling predicts *Tetrastichus* will establish (Appendix F).

Spathius galinae has a biology similar to that of *S. agrili*, however, *S. galinae* originated in the Russian Far East and may complete two or more generations per year. The Russian Far East is more climatically similar to northern regions of North America than to the region of China where *S. agrili* was collected. Thus, *S. galinae* is more likely to establish and has established further north than *S. agrili*. In addition, both *S. galinae* and *T. planipennisi* are more likely to establish in northern regions due to the availability of EAB larvae early in the spring when their adults emerge seeking hosts. For these reasons *S. galinae* is preferentially released in areas where at least 25% of EAB have a two-year life cycle. *Spathius galinae* is expected to fill an important niche because its long ovipositor allows it to parasitize EAB larvae in large diameter ash trees (up to ~23 inches DBH).

REARING EAB PARASITOIDS

The USDA APHIS Biological Control Production Facility in Michigan produces EAB parasitoids for field release. These small parasitic wasps must be reared in EAB eggs or larvae, which are produced or harvested from ash trees felled and removed from EAB-infested woodlots. Although the parasitoids are reared and stockpiled throughout the year for release during the field season, the rearing methods are both time and labor intensive. Research is ongoing on an artificial diet for EAB, but for now fresh logs and leaves are needed for production of EAB and its parasitoids.

The EAB egg parasitoid, *Oobius agrili,* is reared in EAB eggs laid on paper by EAB adults. *Oobius* will be shipped to cooperators either as mature pupae inside EAB eggs on paper held inside pill vials with screening (Oobinators) or rarely as adults in plastic cups with solid caps. *Oobius* pupae are released by removing the plastic cap and attaching the Oobinators to ash trees, with the screen-side down. The *Oobius* adults will emerge and disperse naturally. *Oobius* adults are released from the plastic cups by opening the lids, inverting the cup, and tapping it gently against the trunks of EAB-infested ash trees at release sites.


An Oobinator hanging from a branch.

The three species of EAB larval parasitoid, *S. agrili, S. galinae*, and *T. planipennisi*, are reared in small ash bolts in which EAB larvae are grown from eggs applied to the bark. Although some *Spathius* and *Tetrastichus* adults may be shipped in plastic cups, most of the larval parasitoids are shipped as mature pupae in the small ash bolts. Some of the logs containing *Spathius* species may be waxed (this procedure helps preserve bolt moisture and aids parasitoid emergence). Twine or zip ties are common materials used to attach release bolts to the tree. *Spathius* or *Tetrastichus* adults are released from the plastic cups by opening the lids, removing the screening, inverting the cup, and tapping it gently against the trunks of EAB-infested ash trees at release sites.


Ash bolt with parasitized EAB hanging from a tree.

PROJECT STATUS

Emerald ash borer biological control was initiated more than 20 years ago. During that time, a dedicated group of federal, university, and state scientists completed all the steps necessary for implementation of a successful classical biocontrol project. They conducted host specificity testing, applied for, and received release permits, developed methods for mass rearing, and optimized release methodology. And most importantly are tracking the success of the program. Scientists developed methods for determining parasitoid presence and impact. And a nationwide consortium of cooperators have collected data showing where the various species of parasitoids have established. Over the last 17 years, the EAB Biocontrol Rearing Facility in Brighton, Michigan has produced and released more than 8.5 million parasitoids. Releases have occurred in 32 states (Arkansas, Colorado, Connecticut, District of Columbia, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine,

Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oregon, Pennsylvania, Rhode Island, South Dakota, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin) and 4 Canadian Provinces (New Brunswick, Nova Scotia, Ontario and Quebec).

The first step in evaluating the efficacy of a biocontrol project is to determine if and where parasitoids are being recovered and establishing. Establishment means parasitoids are found at least two years after the final release. *Oobius agrili* has been recovered in 17 states, *Spathius agrili* in 10 states, *Spathius galinae* in 14 states, and *Tetrastichus planipennisi* in 20 states. Recovery does not equal establishment, however, and although *Spathius agrili* can overwinter in northern climates, research has shown that it is not well synchronized with northern weather patterns. It did not establish in any northern states. *Spathius agrili* was collected in Tianjin, China, which is on the coast in the center of the country. The climate in Tianjin is more closely matched with the southern U.S., which could explain why it was unable to establish in the north. A large parasitoid is needed to control EAB in larger ash trees, because *T.planipennisi* is small with a short ovipositor, and cannot attack EAB in trees or branches over 11 cm. Fortunately, another large parasitoid, *Spathius galinae*, was recovered in Vladivostok, Russia, and climate matching indicates it should do well in the northern U.S. *Spathius galinae* has established in many northern states, as have *T. planipennisi* and *O. agrili.* So far, *S. agrili* has only established in Tennessee.

Another important consideration when evaluating the success of a biocontrol program is whether the parasitoids are capable of dispersing away from the release point without human intervention. Several studies have investigated dispersal of the larval parasitoids. Scientists found that *T. planipennisi* was very capable of following EAB populations as they moved, and that this parasitoid can disperse at least 5 km/year. *Spathius galinae* also disperses well, at approximately 7 km/year.

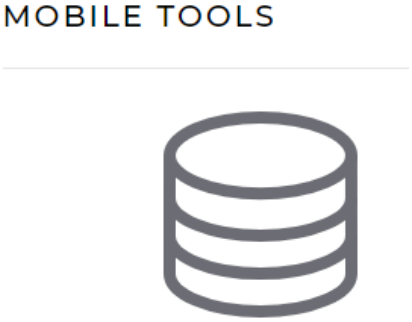
Scientists have been studying the impact of EAB parasitoids on EAB population density since 2014. They have documented an increase in parasitism following release while densities of EAB declined. Sadly, however, when thousands of parasitoids are released in a forest with millions of EAB, especially in the early years of the program when the larger parasitoid *Spathius agrili* didn't establish, most mature ash trees died. For ash to remain a viable component of these forests, it is crucial that the parasitoids can control EAB in small, regenerating ash. Several studies in Michigan and New York show that *T. planipennisi* plays a considerable role in reducing the net reproductive rate of EAB in small, regenerating trees. The parasitoid *S. galinae* was permitted and released beginning in 2015. Recent studies show that it is also having a considerable impact on EAB populations. In these studies, the combination of parasitoids and woodpecker predation leads to the net reproductive rate of EAB falling below one (a net reproductive rate of 1 indicates a population that is neither growing nor

declining).

Recovery of ash populations will occur on the order of decades rather than years. But we are beginning to see signs of ash recovery. Recent assessments of ash regeneration at long term study sites in New York demonstrate that the density of seedling ash at biocontrol release sites did not significantly decline between 2014 and 2021. And the density of larger saplings (>1m) significantly increased. Studies in New England also showed that after release of *Spathius galinae*, the density of healthy ash >12 cm increased from 2015 to 2022. Scientists plan to continue their work to document the impacts of the EAB biocontrol program on pest density and ash health.

PREPARATION FOR PARASITOID RELEASE

This section provides guidance for selecting parasitoid-release sites, collecting data on site characteristics, and releasing the parasitoids. For the EAB Biocontrol Program to monitor and evaluate the establishment of parasitoids and the impact of biocontrol, researchers and cooperators receiving parasitoids from USDA APHIS Biological Control Production Facility **must agree to submit their release and recovery data** to a centrally managed, online, geospatial, searchable database at www.mapbiocontrol.org. **Release data needs to be entered into mapbiocontrol.org within 48 hours after release.** Accurate release and recovery data are critical to the continued success of the EAB Biocontrol Program. And the goal of maintaining ash as a viable part of the American landscape. You must first register to gain access to mapbiocontrol.org. And after you login, you will be asked to agree to enter parasitoid release and recovery data into this central database. This database will store data on where, when, how, under what conditions, and how many parasitoids were released and store data on possible parasitoids recovered. Personnel can use ArcGIS Field Maps (available for Apple and Android) to collect MapBiocontrol release and recovery data. Detailed instructions on using ArcGIS Field Maps are located at www.mapbiocontrol.org under “mobile tools”. Personnel can also use a hand-held GPS device and/or hard copy datasheets to collect data in the field. Data written on hardcopy datasheets and hand-held GPS units must be entered online manually.



The mobile tools button on https://www.mapbiocontrol.org/ where you can find detailed instructions on using ArcGIS Field Maps.

FIELD RELEASE

OUTLINE OF PROCEDURES FOR EAB BIOCONTROL RELEASES

- **Select a release site** in an area with good access, high density of ash trees of various sizes (seedlings, saplings, and mature trees), and infested with EAB, see **Release Site Selection** below for the guidelines on choosing an ideal release site. Please know that APHIS has federal permits to

release all four parasitoids in all states with EAB infestations.

- **Enter Data** about the site location into MapBioControl (www.mapbiocontrol.org). Take site coordinates in the center of the plot where the releases will occur, as described above. Only one release site in a general area is needed to assess parasitoid establishment.
- **Collect General Site Details and Physical Characteristics.** This information is useful for Rearing Facility personnel and state cooperators in prioritizing and selecting the best site(s) for parasitoid release. Enter this information manually on the MapBioControl web site (www.mapbiocontrol.org).
- **Notify the EAB Biocontrol Program** (EAB.Biocontrol.Program@usda.gov) that your site has been submitted to mapbiocontrol.org. All site requests must be submitted by December 1st in the year before you plan to release parasitoids. If you want to release parasitoids in 2025 your request is due by December 1, 2024.
- **Obtain Local Land Use Permits.** Certain locations such as state parks, national forest land, or wildlife refuges may have specific local permit requirements for releasing and/or recovering parasitoids on their land. Be sure to check the regulations applicable to your site.
- **Email Requests for Parasitoids** at EAB.Biocontrol.Program@usda.gov once your site has been approved.
- **Collect Data on Release Trees** (size, EAB density, tree health) using ArcGIS Field Maps on your Phone or tablet or manually enter data online at www.mapbiocontrol.org.
- **Release Parasitoids:** Release at least the minimum recommended number of parasitoids in the spring, mid-summer, and late summer in Year 1 and Year 2 (See Parasitoid Release Section for details). Enter Release data using ArcGIS Field Maps on your phone or tablet or online at mapbiocontrol.org within 48 hours of release.
- **Synchronize Your Phone.** If you collect data in offline mode with ArcGIS Field Maps make sure to synchronize your data once you are back on Wi-Fi to prevent the loss of data or data can be entered manually. Before you can collect data offline, you will need to download an 'offline area' in Field Maps prior to heading out into the field.
- **Assess Parasitoid Establishment:** After completing the releases, you can start assessing establishment in the following year. If releases are done in Year 1 and Year 2 then recovery can start in Year 3. If you do 3 years of releases you would start recovery in Year 4. Yellow pan trap recovery can begin in the spring or summer the year after releases are completed. If you are doing bark sampling or log debarking you can start as early as the fall, winter, or early spring of the year following your releases. Several methods are available for parasitoid recovery, with the choice of method depending on the specific circumstances of each release site. The methods are explained in detail in the recovery section below. It is recommended that you track recovery for two years following your releases so that you can assess if the parasitoids are 'established'. Establishment is loosely defined as observing parasitoids two years after releases have finished. Even if you do not find parasitoids during your recovery efforts, please make sure to enter all recovery data (including zeros) into Mapbiocontrol. This helps create a more comprehensive understanding of where parasitoids are or are not establishing.

RELEASE SITE SELECTION

Although improved rearing methods have allowed for the production and release of greater parasitoid

numbers than in the past, each parasitoid is still costly to produce. Therefore, parasitoids should be released at sites where they have the highest probability of establishment (Appendix F). The site information described below should be collected and entered into mapbiocontrol.org. It will help researchers and the Rearing Facility team determine which sites are most suitable for release and establishment of the parasitoids.

The guidelines below outline the best practices for establishment. Note that these are guidelines to selecting sites and that your site may not have ideal characteristics for each item listed below. However, your site may still be appropriate for releases if it has compensating characteristics or is the most suitable release site for your county. For example, the site may have low acreage, but it has connectivity to other plots. If determining the suitability for a particular site is not straightforward, please email the rearing facility (EAB.Biocontrol.Program@usda.gov) to note that you have found what you believe to be the most suitable release site for your county, even though it doesn't meet all the requirements below.

General Site Characteristics

Locate parasitoid-release sites in naturally forested areas, woodlots, wooded wetlands, and riparian zones. These areas could be within cities or towns but should be of reasonable size. To allow for parasitoid establishment and dispersal, do not select release sites that may be harvested or developed in the next 5 years. State, county, city, and township parks, recreation areas, and game areas are less likely to be disturbed. When possible, avoid sites with excessive human activity as well as sites along roads, trails, railroad tracks, picnic areas, golf courses, or open park lands. This is because ash trees in these areas may need to be removed as they start to decline before parasitoids become established.

Minimum Acreage

Wooded areas at least 40 acres in size are preferred as parasitoid-release sites. Smaller release sites (<40 acres) will require higher ash densities and ash corridors connecting the release sites to other wooded areas. Examples of ash corridors are rivers, ditches, highways, and fence rows. Use of these criteria will facilitate parasitoid reproduction, establishment, and dispersal to nearby areas.

Relative Density of Ash

If possible, at least 25% of the trees should be ash, but a higher percentage of ash would be even better. The percentage of ash can be estimated as ≤25%, 26-50%, 51-75%, or 76-100%.

Ash Tree Size Class

Ideally, parasitoid-release sites should contain a variety of ash size classes ranging from seedlings to mature trees. Older and highly stressed ash trees in a stand are generally attacked first by EAB and tend

to die off more quickly. Although these trees are less likely to benefit from EAB biological control, they will provide a high density of EAB eggs and larvae, increasing the probability of parasitoid reproduction at the site. Smaller trees, saplings, and seedlings provide potential for regeneration of ash trees, and will support EAB and their natural enemies following the loss of larger ash trees in the stand. *Tetrastichus*, which has a short ovipositor, appears most likely to establish in areas with some smaller, thin-barked ash trees, where EAB larvae are more accessible.

Density of EAB and Health of Ash Trees

Low to moderate EAB-population densities are recommended for potential parasitoid-release sites. Unless there are many young trees in the vicinity, stands with dead and dying ash trees are not appropriate as release sites because ash and EAB may decline or crash before the parasitoids become well established. The most accurate method of estimating EAB density requires felling and peeling the bark from ash trees to count EAB present under the bark and along the trunk. This is a direct estimate of EAB density, however, is difficult, labor intensive, destructive, and counter-productive in areas where EAB density is low. Therefore, after EAB has been confirmed at a site, it is okay to use an indirect EAB-density estimate based on the signs and symptoms of EAB infestation in the ash trees.

During the winter, before spring leaf flush, the most apparent symptom of EAB attack on the trunks of ash trees is woodpecker feeding and sometimes bark scaling. As EAB densities increase, EAB symptoms readily visible lower on ash trunks include bark splits, D-shaped EAB adult exit holes, epicormic shoots, and stump sprouts (Appendix A). Symptoms of dead ash trees include bark that is falling off trees, leaving exposed galleries and D-shaped exit holes (Appendix A).

During the spring and summer when the trees have leaves, the condition of ash trees can be visually ranked according to the five crown-condition classes illustrating typical EAB- induced decline; crown condition 1 is a healthy canopy, 2, 3, and 4 show increasing decline, and 5 is dead crown (Appendix D). Overall, ash trees at a potential release site should be fairly healthy, with an average crown condition of 1 to 2 (healthy or mostly healthy) and only a few trees in condition classes of 4 to 5 (dying or dead). Other insects and diseases can cause ash canopy decline, epicormic sprouts and/or woodpecker feeding. Therefore, the presence of EAB must be confirmed at each potential release site. This is done by selecting ash trees with signs of stress from a possible EAB infestation. On these potentially infested trees, remove sections of bark using a chisel or draw knife to confirm the presence of EAB galleries or EAB life stages (Appendix A). However, early in the EAB outbreak cycle when the density of EAB is low, most EAB will be higher on the trunks, thus confirmation may require felling and debarking ash trees in the stand.

The one exception to selecting sites with low to moderate EAB-population densities is sites that would be considered aftermath sites. This would be counties in the northern U.S. where *Spathius agrili* did not

establish which are now eligible for release of *Spathius galinae* or counties where most of the mature trees have already died but there were no parasitoid releases previously done. These aftermath sites must have plenty of live small ash trees available for EAB and its parasitoids for establishment to occur. Basal sprouts and saplings need to be over 1 inch in diameter to support EAB.

Access and Permissions

Select release sites at locations that are relatively easy to access because personnel will need to visit the site periodically for parasitoid release and recovery activities. Obtain permission and/or permits from the landowners or park managers for use of the site to both release parasitoids and conduct recovery activities over a period of five years. Certain areas like state parks, national forest land, or wildlife refuges may have specific permit requirements for releasing and/or recovering parasitoids on their land. It may take months to obtain permission or land-use permits from landowners or park managers.

PRE-RELEASE SITE ASSESSMENT

Prior to requesting parasitoids for release, we recommend collecting some preliminary data on site characteristics that will help the Biocontrol Rearing Facility staff assess whether your site is appropriate for parasitoid release. Note, with the new ArcGIS Field Maps app you cannot collect any data for your location until your release site is approved by APHIS. To collect General Site Details and Physical Details data you will need to enter it manually online at www.mapbiocontrol.org. When collecting your general site details be sure that you are at the location where parasitoids will be released. Do not collect GPS coordinates next to the road. Ideally the parasitoids should be released in the center of the forest, or at least 100 m from the road or other non-forested areas. Once the data is collected, Rearing Facility personnel can review the site and determine if the site is appropriate for release. The information provided, including location, size (number of acres or hectares), percentage ash, and EAB density will assist Rearing Facility personnel in prioritizing and selecting the best site(s) for parasitoid release.

To enter data about a new **Release Site** into MapBioControl, click on "Release" in the green banner. Click the "New" button in the upper gray table, and then enter the following data:

- **Status:** Select "Proposed" because the site has not yet been approved.
- **State**
- **Date**
- **Site Name**
- **Site Location:** Enter general information such as county, town, park name, address, major roads, etc.
- **Latitude** (dd.dddddd)
- **Longitude** (dd.dddddd)

- **Plot** (whether it is a release or control plot)
- **Type** (program or research)

To continue entering data about your new site, click on the site in the upper table to highlight it (it will turn yellow). Then click on one of the tabs below. When you click on the General Details Tab or the Physical Details Tab you will need to highlight the line of blanks in the lower table (it will turn yellow) before you can click on the "Edit" button to enter the data. To see more information about each entry item hover the mouse over the category. Enter site characteristics data as follows:

General Details

- **Size of wooded area in acres** (you can use the measurement tools with Google Earth or ArcGIS Explorer)
- **% ash** (estimate: <25%, 25%, 50%, 75%, 100%)
- **Dominant Tree Species**
- **2nd most Dominant Tree Species** (if applicable)
- **3rd most Dominant Tree Species** (if applicable)
- **EAB Density** (Low, Medium, High)
  Low: EAB present but difficult to find.
  Med: Trees are beginning to show signs of EAB infestation.
  High: >25% of trees show signs of EAB infestation.

Physical Details

- Topographic Position (Upper Slope, Mid Slope, Lower Slope, Level)
- Flooding (Dry all year, Seasonally Wet, Wet all Year)
- Degree of Isolation (Surrounded by non-woodland or connected to other woodlots)

RELEASE OF PARASITIODS

RELEASE CONSIDERATIONS: WHICH SPECIES, WHERE, WHEN

Prior to 2012, the EAB Biocontrol Program provided *S. agrili, T. planipennisi, and O. agrili* to each state for release upon request. However, we now know that the establishment of all three parasitoids varies with geographic area, and they are not all suitable for releases in all states. Thus, we have made new recommendations based on current research results (Appendix F).

In theory, a higher number and frequency of parasitoids released increases the probability of establishing stable parasitoid populations. In reality, the number and frequency of parasitoid releases are often limited by the resources that are available for parasitoid production. The minimum numbers of parasitoids recommended for release are listed below by species. The actual numbers shipped may vary depending on total availability during any given week and the number of release sites requiring insects. Whenever excess parasitoids are available, they are often added to the original number of

parasitoids that were requested. Each release will consist of a specified number of female parasitoids, although males are also included in shipments of adult parasitoids (for larval parasitoids only). Considering that weather patterns in any given year can impact the synchrony between availability of the appropriate stages of EAB and release timing, and severe weather events may reduce parasitoid survival, releases should be made during two consecutive years at each biocontrol release site.

Larval parasitoids should be released when late-instar EAB larvae (3rd- and 4th-instar larvae) are present in the field. Making firm recommendations on release timing is difficult because EAB larval development is variable and depends on factors such as when the eggs were laid, temperature, and ash tree health. In addition, *Oobius* should be released when EAB eggs are present. However, EAB eggs are very small and nearly impossible to find in the field, and EAB larvae are under ash bark and are not accessible without peeling trees. Spreading releases out over multiple weeks helps ensure that the proper stages of EAB eggs and larvae are present for parasitism. Growing degree day accumulations and forecasts can be found at http://uspest.org/US/ and http://uspest.org/cgi-bin/ddmodel.us. Growing degree days are used to estimate EAB larval development and help the rearing facility to decide when to start releases in each state.

Below is a discussion of when and where to release each parasitoid species.

Tetrastichus planipennisi

- *Tetrastichus planipennisi* will be preferentially released at locations that accumulate fewer than 3,500 GDD50F between January 1 and September 30. This corresponds to the part of the United States where 26-80% of EAB overwinter as larvae (close to the surface of the tree) rather than as J-larvae (in pupal chambers in the wood) and are available for parasitism in the spring. We use a model that delineates the areas expected to have over 25% EAB overwintering as larvae shown in Appendix F.
- If your site accumulates > 3,500 GDD50F in the summer and you would still like to release *T. planipennisi* please contact the rearing facility. You will need to confirm that you have 3-4th-instar EAB larvae in late winter or early spring before scheduling *T. planipennisi* releases. If between 3,500 and 3,975 GDD50F accumulate at your site between January 1 and September 30 then you can expect to have between 11-25% of EAB overwintering as larvae.
- We rarely see establishment of *T. planipennisi* at sites where >3,975 GDD50F accumulate in the summer, and we do not recommend releasing this species in these locations.
- *Tetrastichus planipennisi* has a short ovipositor. Make sure there are plenty of trees and branches less than 5 inches in diameter at your site that contain EAB larvae before releasing. Saplings and branches need to be over 1 inch in diameter to support EAB.
- Initiate releases in the spring after 300 GDD50F have accumulated.
- Release again in the late summer-fall when mature larvae are available, generally between 1400

and 2500 GDD50F.

- If you do not have any mature larvae in the spring, do not release *T. planipennisi* in the fall; there will be nothing for them to parasitize when they emerge the following spring.
- Release at least 200 females two weeks apart for 6 weeks (a minimum of 600 released per season – 1,200 per year).

Spathius galinae

- Counties in the northern U.S. where parasitoids, including *S. agrili*, were released but where *S. agrili* did not establish are eligible for release of *S. galinae*. A parasitoid with a longer ovipositor is needed to attack EAB in larger ash trees.
- Sites where the large ash trees have died are still suitable for release of *S. galinae* because it can attack EAB in smaller trees. But there must be plenty of live small ash trees available for EAB and its parasitoids for establishment to occur. Basal sprouts and saplings need to be over 1 inch in diameter to support EAB.
- *Spathius galinae* also emerges early in the spring and will only be released following the same guidelines as for *T. planipennisi*.
- Initiate releases in the spring after 300 GDD50F have accumulated.
- Release again in the late summer-fall when mature larvae are available (generally between 1400 and 2500 GDD50F). If you do not have any mature larvae in the spring, do not release *S. galinae* in the fall; there will be nothing for them to parasitize when they emerge in the spring.
- Release at least 100 females two weeks apart for 6 weeks (a minimum of 300 released per season – 600 per year).

Spathius agrili

- *Spathius agrili* will only be released at sites that accumulate more than 3,500 GDD 50F. It has not been established in areas with fewer GDD.
- Initiate releases when mature 3rd- and early 4th-instar EAB larvae are present in ash trees. That could be as early as mid-June (in southern states such as Arkansas) or in mid-July in more central states such as Tennessee. We do not yet have degree day estimates for initiating releases and scraping some bark to find EAB and determine instars prior to release is recommended.
- Release at least 200 females per week for 6 weeks (1,200 total).
- Do not conduct any releases after the end of August.

Oobius agrili

- *Oobius agrili* can be released in all states.
- Initiate releases at 600 GDD50F. Release at least 200 individuals per week for 3 weeks (600 total).
- Initiate the second round of releases at 1400 GDD50F. Release at least 200 individuals per week for 3 weeks (600 total).
- Releases can continue until ~2,500 GDD50F have accumulated.

REQUESTING PARASITOIDS

Email all parasitoid requests to the EAB Biocontrol Program mailbox (EAB.Biocontrol.Program@usda.gov). To be eligible for release you must submit your detailed site information to Mapbiocontrol and submit a request to EAB.Biocontrol.Program@usda.gov. If you have question about site approval or what parasitoids to request, you can email EAB.Biocontrol.Program@usda.gov. All site requests must be submitted by December 1st in the year before you plan to release parasitoids. So, if you want to release parasitoids in 2025 your request is due by December 1st, 2024. Release sites in new states and counties will be given top priority.

RECEIPT OF PARASITOIDS

In the case of an emergency in receiving or deploying parasitoids, please contact the EAB Biocontrol Program mailbox (EAB.Biocontrol.Program@usda.gov), Scott Whitehead (Biological Control Release Coordinator, 810-844-2708) or Nicole Sawallich (Biological Science Technician, 810-844-2704). Parasitoids are shipped by overnight delivery in a cardboard box and should arrive by 10:30 AM Eastern Time at most locations. *Spathius* and *Tetrastichus* will be shipped either as developing pupae inside ash bolts or as adults in 16-oz plastic cups with screening on the lid. Twine, rope, and zip ties are commonly used to attach bolts to the trees but are not included in parasitoid shipments. *Spathius galinae* bolts will be waxed, and *Tetrastichus* bolts will not be waxed. Wording on the top of the bolt should indicate species and how many adults are expected to emerge from that bolt. Honey will be smeared on the lid as a source of food for the adult parasitoids in cups. Rarely adult *Oobius* will be shipped in plastic cups with honey streaked on the walls of the cup and sealed shut with a snap-top plastic lid lined with filter paper. *Oobius* will most commonly be shipped as pupae inside EAB eggs on paper held in small plastic vials that can be hung on ash trees at release sites. **Be sure to remove the cap before deploying the vial so that the emerging parasitoids can escape.** Small twist ties are fixed to these containers for hanging on small diameter branches, but this apparatus can also be attached to tree boles or larger diameter branches with twine, zip ties, or aluminum nails.

The parasitoids should be released soon after receipt.

When possible, all parasitoids should be released on the day they are received. If you are unable to release parasitoids as scheduled because of an emergency, contact the Biological Control Release Coordinator as soon as possible. After arrival, transport the parasitoids in the container to the release site. *Oobius agrili* do not fly as far as *Tetrastichus* and *Spathius*, so their release cups should be spread throughout the stand to enhance their establishment and dispersal.

CARE OF PARASITOIDS IF RELEASE IS DELAYED

The parasitoids should be released on the day of arrival, however, if there is an unforeseen delay

caused by late delivery or unexpected weather conditions, place the cardboard containers in a room that does not become overheated, unseal, and open each box and internal plastic bag to determine the contents. Boxes containing:

- Ash bolts with immature larval parasitoids can be held in closed bags and boxes.
- Immature *Oobius* pupae on paper inside small plastic vials with screening will require you to open the box and the plastic bag and keep them where they will not become overheated.
- Adult parasitoids inside clear plastic shipment cups, will require your care to survive beyond the day of arrival.
- To care for adult larval parasitoids (*Tetrastichus* and *Spathius*), open the box, remove, and open the bags. Inside each bag will be several labeled cups containing small groups of live parasitoids. To maintain sufficient ambient moisture for the parasitoids, we recommend placing the rearing cups in a clear plastic storage tub with moistened paper toweling. If the delay until you can release the parasitoids will be over 24-36 hours, you can check each cup for the presence of honey. Honey provides the parasitoids with food and some moisture during shipping. If no honey is visible on the screening on the lids of the cups with *Spathius* or *Tetrastichus,* put two or three drops of honey on the lid and gently smear it. Open lids very carefully so adults do not escape.
- *Oobius* adults shipped in clear plastic cups do not require additional honey and should not be opened. The cups should be held in open coolers in a well-lit room where they will not become overheated, as described above for the immature *Oobius* on paper in vials.

TRANSPORTING PARASITOIDS TO FIELD SITES

Carry the cups or infested logs inside the boxes when transporting parasitoids to the field for release. For delayed releases they do not need to be re-bagged for local transport. Care should be taken to keep the box out of direct sunlight or other potentially hot (e.g., a sealed vehicle) environments. Parasitoids are extremely sensitive to overheating when confined. The trunk of a vehicle will suffice, but an air-conditioned interior is even better, provided the vehicle will not be allowed to sit unattended in the sun for any period. **Keep the box in the shade** because parasitoids are extremely sensitive to overheating when confined. Some people have stored parasitoids under their car to ensure shade, but this should be done with caution since a car may be extremely hot near the engine. Keep the box closed except to remove the cups or logs with parasitoids for release. Carry the box carefully and avoid sudden movements. Adult parasitoids are susceptible to drowning in droplets of water or honey if the cup is inadvertently shaken or dropped.

RELEASE OF PARASITOIDS

Adult Parasitoids

If possible, release the parasitoids in the morning or evening so they can move about in the

environment before the onset of high afternoon temperatures. Gently tap the cup before opening, this allows the adult parasitoids to drop down to the bottom of the cup and avoids injuring any that are gathered near the lid as you open the cup. Carefully remove the snap-top lid with the tissue-paper liner or fabric liner when opening cups containing *Oobius, Spathius* or *Tetrastichus* adults. Place the cup and tissue next to the trunk of an appropriate ash tree. On warm sunny days, most of the parasitoids will crawl up to the lip of the cup onto the tree trunk or simply fly away. On cooler days, most of the parasitoids will remain in the cups. To dislodge these parasitoids, hold the cup upside down at a slight angle against the tree trunk and gently tap the cup against the tree, causing the parasitoids to jump or fly onto the tree trunk. You may find a small paintbrush useful for gently guiding any *Spathius* or *Tetrastichus* parasitoids that refuse to leave the cup. Move the cups from tree to tree to ensure the number of each species is somewhat evenly distributed throughout the release site, this is especially important for *Oobius* as they do not fly as far as *Spathuis* or *Tetrastichus*.

Larval Parasitoid Pupae in Small Ash Bolts

The bolts containing parasitoid pupae will need to be attached to the trunk of the tree. Twine, zip ties, wire, or aluminum nails are commonly used to help hang the bolts on the trunk or branch. These materials will need to be purchased because they are not provided by the rearing facility. Care should be taken to try to minimize direct contact between the bolt and the tree trunk because parasitoids can emerge from all sides of the bolt. Do not hang more than one bolt per tree if possible; the parasitoids will establish better if they are spread out. Choose trees that are alive, trees with signs of active EAB infestation are preferable to trees with no signs of EAB infestation. Bolts should be labeled on the top by which species they are and how many females are in each log so you can spread out your releases of each species. You will also notice that bolts containing *Spathius galinae* have been waxed to improve the emergence of this species. The bolts should remain in the field for at least six weeks to ensure that all the parasitoids have emerged as adults. **If using nails, please only use aluminum nails or remove the nails from the trees when you recover the logs because nails will harm sawmill equipment if the trees are harvested.**

***Oobius* Pupae inside EAB Eggs on Paper in Oobinators**

Hang one Oobinator per ash tree and distribute them widely throughout release sites to encourage the spread and dispersal of *Oobius*. Please choose trees that have evidence of fresh EAB attack such as current woodpecker feeding, live epicormic shoots along the trunk, or moderate canopy dieback (classes 2-3). Ash trees with flaky or coarse bark are preferred because they provide more oviposition sites for EAB to lay eggs; these are often the larger-diameter trees in the stand. Each oobinator must be

left on the trees for at least six weeks to allow all *Oobius* adults to emerge. **Note: The oobinators in your *Oobius* shipment will have caps, do not forget to remove the caps before hanging them in the field.**

ENTER RELEASE DATA

Release data provides valuable information on establishment of these parasitoids. Please record/write down this data the day you conduct your releases. **We ask that you enter release data using the ArcGIS Field Maps app on your iPhone/iPad or online at mapbiocontrol.org within 48 hours of release.** To estimate the number of parasitoids released you can use your parasitoid invoice. The tops of ash logs will also have a number that shows an estimate of females in that log. Oobinators and cups of adults have the number of females written on the side or top. Every time you release parasitoids, enter the following information into your ArcGIS Field Maps app and synchronize/upload it, or these data can be recorded on a datasheet in the field (see Appendix G) and then entered directly online in mapbiocontrol.org:

- **Release Date**
- **Release time**
- **Weather Conditions** (Sunny, Partly Cloudy, Foggy, Light Rain, Moderate Rain, Heavy Rain, Thunderstorms)
- **Wind Speed** (Light, Moderate, Strong)
- **Temperature** (Degrees Fahrenheit)
- **Number of Female *Oobius* Released** (when releasing as pupae this will be an estimate)
- **Stage *Oobius* released** (Adult, pupae, both)
- **Number of Female *Spathius agrili* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Spathius agrili* Released** (Adult, pupae both)
- **Number of Female *Spathius galinae* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Spathius galinae* Released** (Adult, pupae both)
- **Number of Female *Tetrastichus* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Tetrastichus* Released** (Adult, pupae, both)
- **Notes**

EVALUATING PARASITOID ESTABLISHMENT

Several methods have been developed that can successfully recover the four parasitoids released for EAB biocontrol. Unfortunately, none of the methods is consistently more effective than the others, and there are circumstances where parasitoids are recovered using one method but not others. Yellow pan traps are inexpensive and easy to sample, but they do not give any indication of the number of EAB

attacked by parasitoids. Collecting EAB eggs and larvae from trees allow us to calculate percentage parasitism. However, these methods may require felling the tree for an adequate sample of EAB larvae from under the bark to detect larval parasitism. If resources permit, the best option is to use a variety of methods to ensure that if the parasitoids are present, you can recover some.

All recovery efforts should be started in the year after releases are finished. If releases are done in Year 1 and Year 2 then recovery can start in Year 3. If you do 3 years of releases you would start recovery in Year 4. Yellow pan trap recovery can begin in the spring or summer the year after releases are completed. If you are doing bark sampling or log debarking you can start as early as the fall, winter, or early spring of the year following your releases. It is recommended that you track recovery for two years following your releases so that you can assess if the parasitoids are 'established'. Establishment is loosely defined as observing parasitoids two years after releases have finished. You only need to sample one release site per county, although more is always better. Below we describe the four parasitoid species and how their life cycle affects recovery sampling:

Tetrastichus planipennisi is a gregarious endoparasitoid (internal parasitoid) of EAB larvae, and 20 to >100 *Tetrastichus* larvae can develop inside their host. *Tetrastichus* may have three to four generations per year. An EAB larva parasitized by *Tetrastichus* may 1) look healthy; 2) appear lumpy like a "braided rope"; 3) be replaced by a mass of small grub-like larvae (white), pupae (color ranges white to bluish-black) and/or adults (dark metallic blue); or, 4) emerged from the EAB gallery, leaving only the head and tail of the EAB larva and small black spots in the gallery (the spots are waste excreted by each *Tetrastichus* larva before pupation is complete) (Appendix B). These parasitoids pupate in the EAB gallery and may be recovered by debarking ash trees. Adults may be recovered in yellow pan traps.

Spathius agrili **and** ***Spathius galinae*** are gregarious ectoparasitoids (external parasitoids) of EAB larvae, and all life stages live on the outside of the host. *Spathius* eggs and small larvae are difficult to see with the naked eye, but by late fall, most will be large larvae or will have spun silken cocoons that are fairly easy to see in the EAB galleries (Appendix B). Like *Tetrastichus*, adults of *S. agrili* and *S. galinae* pupate in the EAB gallery and may be recovered by debarking ash trees. Adults may be recovered in yellow pan traps. There are several native species of *Spathius* so identification of larvae and pupae recovered requires DNA testing, identification of adult *Spathius agrili* also requires DNA testing.

Oobius agrili spends the winter in diapause inside EAB eggs, which are difficult to find sheltered between layers of bark and in bark crevices. EAB eggs are light brown or gold, whereas *Oobius*-parasitized eggs are often darker brown in color (Appendix B). Ash bark with EAB eggs can be scraped off trees in the field without injuring the tree, and the bark is dried and sifted to recover EAB eggs in the laboratory. Selecting trees that are more likely to have EAB eggs is important, see the section TREE FELLING AND DEBARKING below for more information. Although *Oobius* adults are small, they can also be recovered in yellow pan traps.

NOTE: If you would like examples of parasitoid adults, larvae, pupae, or cocoons to help with identification of parasitoids from the field, please contact the Rearing Facility for specimens at EAB.Biocontrol.Program@usda.gov. If you have questions about parasitoid identification, recovery methods, where to purchase supplies, or how to construct yellow pan traps or emergence tubes, please email EAB.Biocontrol.Program@usda.gov.

YELLOW PAN TRAPS

Many insects are attracted to the color yellow. Parasitoid recovery studies have shown that yellow pan traps (YPTs) are effective at trapping the introduced EAB parasitoids *Tetrastichus planipennisi, S. agrili, S. galinae,* and *O. agrili*. Other known EAB larval parasitoids (e.g. *Atanycolus,* native *Spathius* spp., *Phasgonophora sulcate* and *Balcha indica*) are also trapped, along with many other species of bees, wasps, flies, plant hoppers, and beetles. YPTs are simple and inexpensive to make.

Yellow Pan Trap Set-up Considerations

What makes a good trap tree for hanging a yellow pan trap?

Over the years we have found yellow pan traps work really well in some situations and not so well in others. While ongoing research continues to unfold, our initial insights are as follows. Successful parasitoid recovery in YPTs seems to depend on where they are placed, and some trees are better for recovering parasitoids than other trees. Often, we find the same trees will catch parasitoids week after week, while nearby trees will recover nothing. We are not sure yet exactly what makes an ideal tree which is why we recommend placing multiple traps. We have been investigating where the yellow pan traps are most successful and current thinking is that they might work better in areas where there is ash along the edge of the forest (some of our most successful sites have been along greenways or bike paths or in street trees). Overall trees within a site that get more sunlight are preferred. Parasitoids fall into the traps as they search for EAB on the trunks of the trees and larval galleries are more common on the south and west facing side of sunny host trees. Research has shown consistently that trees with

visible signs of fresh EAB and live phloem are the best for catching parasitoids (woodpecker feeding on the lower half of the tree is correlated with higher trap catches). This makes sense because parasitoids are going to look for hosts where hosts are present. Preliminary research shows that moderate to high canopy dieback (10-80%) is suitable for catching larval *Tetrastichus planipennisi* and likely *Spathius* spp., and that moderate dieback (10-30%) is preferable for *Oobius agrili*. Trees with a large number of fresh woodpecker holes are preferable and canopy dieback is of secondary importance to finding fresh woodpecker damage and live phloem. Due to the dynamics of our forested areas, it is not always possible to find "ideal" trees, and a given tree will only be suitable for hanging yellow pan traps for a few years. Each year just do the best you can to find live but infested trees on which to hang the traps, choosing suitable trees is very important to the success of yellow pan trapping.

How many YPTs should I deploy and where?

Deploy a total of 15 YPTs with one YPT per ash tree at your EAB biocontrol release site. If possible, select a variety of sizes of ash on which to place the bowls. *Tetrastichus planipennisi* cannot parasitize EAB in ash larger than 4 ½ inches, EAB (and thus Oobius) are more likely to be found when the bark is flaky (as in larger trees), and *Spathius* species should be found on trees of varying sizes. The trees you select must show some symptoms of EAB infestation (e.g. fresh woodpecker feeding, epicormic shoots) with crown class 3, or 4. Do not put the traps on dead or healthy ash trees (parasitoids will not be foraging on these trees). Distribute YPT's on ash trees throughout the release areas and, if possible, place some traps at or near the release trees.

Label each YPT holding bowl with a unique ID number using a weather-proof pen (e.g. Sharpie) or grease pencil if bowl is wet. On a data sheet, record the YPT-ID number, date, and initial of person collecting. Record the GPS coordinates – this will help you find the YPT later to recover the sample, and it will let researchers know where the parasitoids were recovered.

Please know that trapping can generate a substantial sample load that will need to be processed. Samples can be frozen until processed later. If staff hours are limited, you may want to consider fewer sample dates or fewer sites. We do not recommend putting out fewer traps.

When to deploy YPTs in the field?

Deploy YPTs at EAB biocontrol release sites in the year after your final parasitoid releases. If you finish releases in August, you can start YPT recovery the following April or May. *Tetrastichus planipennisi* and *Spathius galinae* fly during the spring, summer, and early fall. *Oobius agrili* is active when EAB is laying eggs. *Spathius agrili* does not emerge until 4th instar EAB are available (late June-July depending on site climate), and if you did not release *T. planipennisi* or *S. galine*, there is no need to put out traps in the early spring. All four introduced parasitoids have multiple generations per year.

Thus, their populations are highest in mid-summer and early fall. However, a few studies have found the highest captures for larval parasitoids *Tetrastichus planipennisi* and *Spathius galinae* occurred in the spring. Overall, the timing of possible captures in YPTs will depend on the climate in your area. And the adult flight period of each species.

When to deploy YPTs for Tetrastichus planipennisi and/or Spathius galinae?

If you can do intensive sampling, we recommend deploying the YPT by 200 Growing Degree Days (GDD) base 50F. Then throughout the spring, summer, and early fall until 2900 GDD_{50F} (Or 110 to 1600 GDD base 10C). This requires visiting sites weekly, collecting the samples, and replacing the liquid in the bowl for the next week of sampling. If you can't do intensive sampling, then we recommend starting sampling by 200 GDD_{50F}. And sampling for at least 6 weeks.

When to deploy YPTs in the south?

Where larval parasitoids are not expected to emerge during the spring, deploy the YPTs around 720 GDD base 50F (400 GDD base 10C) for *Oobius*. Or when 3rd-instar larvae become available in the mid-late summer for *Spathius agrili*. That could be as early as mid-June (in southern states such as Arkansas). Or in mid-July in more central states such as Tennessee.

When to deploy YPTs for *Oobius agrili?*

If you are only sampling for *Oobius* we recommend deploying the YPT by 720 GDD base 50F. And trapping until 2200 GDD_{50F} (or 400 to 1200 GDD base 10C).

How long do I leave YPTs in the field?

The YPT samples should be collected five to seven days after the new liquid has been placed in the trapping bowl. Samples left too long in the field will decay or dry up. Seven days is ideal because the longer the traps remain in the field, the more likely they will trap one of the target parasitoids. If you anticipate a heavy downpour, however, you might want to consider collecting the samples early. In limited cases, cooperators have found that if they increased concentrations of propylene glycol to 50%, samples could go up to 2 weeks before collecting. Care must be taken to test that this method will work at your site, if you find samples drying out or decaying, restart weekly collections. When servicing traps collection bowls must be filled to the overfill hole to prevent it from drying out. In addition, if you get a heavy rain event, empty traps before or soon after because rain will both displace the solution from the bowl and dilute the remaining solution which can lead to rapid decay of insect specimens.

Yellow Pan Trap Building & Deploying Traps

What will I need to make and deploy one YPT?

1. Two 12-oz, 7–8 inch bright yellow plastic bowls
2. One 8-inch (8 by 6 or 8 by 10) right-angled shelf-bracket.

3. Three 1.25-inch-long wood screws.
4. Two small binder clips for securing the collection bowl to the holding bowl.
5. A one-hole punch and a utility knife for altering the bowls.
6. Fine-mesh screening (e.g. organza). If purchasing, try searching for no-see-um mesh.
7. A hot glue gun.
8. Weather-proof marking pen (e.g. Sharpie) or grease pencil (needed if bowls are wet).
9. Three 6-inch zip-ties (make sure they are thin and fit through the holes on the shelf bracket).
10. 20% solution of **clear** (not pink or green) **Food grade** and/or **USP grade** propylene glycol propylene glycol (non-toxic antifreeze) diluted with water. It is easiest to by pure propylene glycol and dilute it yourself.
11. Rechargeable portable electric screwdriver with bit and extra battery pack.
12. Unscented clear dish detergent

For detailed instructions on how to construct yellow pan traps, please email EAB.Biocontrol.Program@usda.gov.

What will I need to collect the insect sample from the YPT?

1. One paint filter per pan trap per sample occasion (We use 190-micron fine mesh paint filters).
2. One Zipper or whirl pak plastic bag per pan trap per sample occasion (We use 6" by 9" bags).
3. Pencil (Not pens) and paper.
4. 20% solution of **clear** (not pink or green) **Food grade** and/or **USP grade** propylene glycol propylene glycol (non-toxic antifreeze) diluted with water. It is easiest to by pure propylene glycol and dilute it yourself.
5. Unscented clear dish detergent.

How are the YPTs mounted?

Using the electric screwdriver, attach a shelf bracket to the trunk of a **living ash tree infested with EAB.** Attach the bracket ~5 feet above the ground with the three wood screws. Make sure the top bracket is level or the bowl will not sit properly, you may have to leave the top screws partially unscrewed so that the bracket doesn't tilt up too far up.

What about those two yellow bowls?

One yellow bowl is used as a "holding bowl." It is attached to the 8" side of the shelf-bracket with zip-ties threaded through the three shelf-bracket holes (on the horizontal surface). The zip ties should be threaded through pairs of holes punched into the holding bowl with a paper punch (0.5 to 1.0 cm below the lip) and then through the hole in the shelf bracket. There are two holes in the shelf bracket next to the tree and one hole at the tip. Do not pull zip-ties too tightly to avoid distorting the holding-bowl. To

provide drainage in the holding- bowl, cut a hole or two (~2.5-cm square) in the bottom with a utility knife.

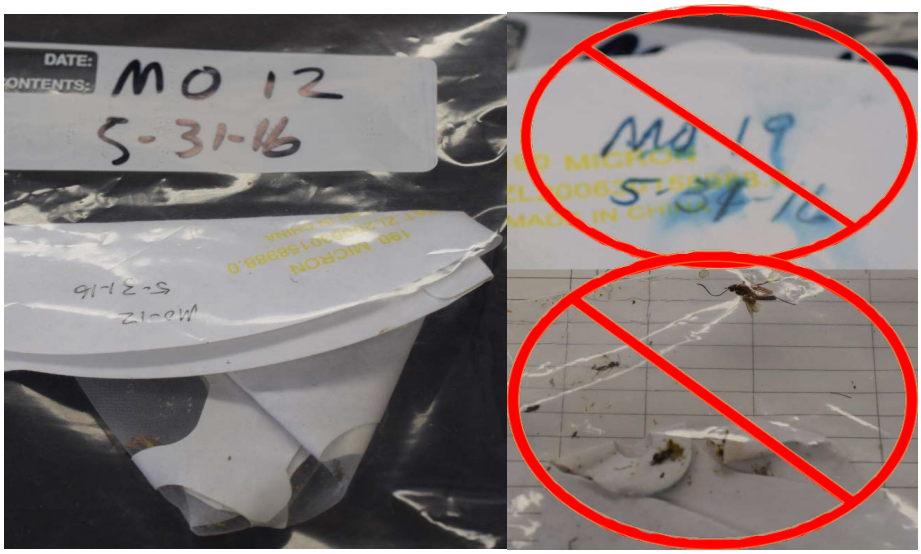
A collection bowl being put into the holding bowl of a yellow pan trap. Photo by J. Hansen.

The second yellow bowl or "collection-bowl" will hold the liquid that traps insects. It rests inside the holding-bowl. To prevent overflow from the collection-bowl after rainfall, punch at least 6 drainage holes just below the lip. Hot-glue a strip of fine-mesh screening (e.g. organza) over the drainage holes to prevent loss of specimens during overflow. After the bracket and holding-bowl are mounted on the tree, set the collection-bowl in the holding- bowl. Fill the collection-bowl ~¾-full with the 20% propylene glycol solution (make sure that the propylene glycol is clear, not pink). Add one drop of unscented dish detergent to break the surface tension of the solution. This will allow inquisitive insects to become entrapped in the liquid. You will need to empty the collection bowl after three to seven days to avoid possible loss of the sample due to weather, vandals, wildlife, decay, etc. We find that it is most convenient to collect samples once per week, adding fresh propylene glycol after collecting the first sample and continuing weekly samples. Secure the bowls together using two binder clips to prevent the bowl from getting blown out by the wind, which can happen as the liquid level lowers during drier weeks.

How is the insect sample collected from the YPT?

After locating the YPT in the field, label your paint filter with the state, site, date (including year), and pan trap # in pencil (pen or marker may wash out). If there are many leaves in the sample, swish them in the liquid to dislodge any insects and discard them. Consider doing this with slugs as well as their slime can make the sample gummy and hard to process. Pour the contents of the trap (insects plus liquid) through a paint filter. A squirt bottle with water is recommended for dislodging insects from the bottom of the bowl or from leaves/slugs. The propylene glycol is not toxic and can be poured on the ground.

Fold over the open end of the paint filter and place each one separately into a zippered bag. If the filter is labelled in pencil, you do not need to label the bag. If you cannot label the filter paper (because it is too wet, for example) then label the bag instead with Sharpie. Consider bringing a cooler with ice packs while collecting samples if it is hot to help preserve insects before they are processed. A few hours in a hot car can cause insects to start to disintegrate and make it harder to ID samples later. Store samples in the refrigerator and process them within one or two days. If processing is delayed, store the samples in the freezer.



Please label the filter paper in pencil to avoid ink running and ruining the label. Also fold the filter paper over to avoid insects spilling out prior to processing. The photo on the left shows the best practice for collecting a yellow pan trap sample. The photos on the right shows how the ink can smudge and insects can escape if a sample isn't labelled in pencil and folded at the top.

What do I do with these samples?

Because so many states are now doing recovery sampling, APHIS does not have the resources to sort potential parasitoids from YPT samples. Processing, or sorting, the YPT samples should be done locally, and suspect insect parasitoids that resemble the EAB parasitoids must be sent for positive identification because several native parasitoids are easily mistaken for the EAB parasitoids. Identification of these parasitoids requires specialized taxonomic skills. To assist you in sorting the insects in the YPT samples, please contact the rearing facility (EAB.Biocontrol.Program@usda.gov) for examples of adult males and females of each EAB parasitoid species and refer to the instructions below.

Processing Yellow Pan Trap Samples

Before processing or sorting the YPT samples, please contact the rearing facility for examples of adult males and females of each EAB parasitoid species. These will be helpful, along with the photos below, for you to find and identify possible EAB parasitoids in each YPT sample. It is especially important

that you look at the example specimens using the same magnification you will use to sort the parasitoids from the YPT samples. Even though the parasitoids are quite small, you will be surprised at how large they look under magnification. Once you have prescreened your samples, send all suspect specimen samples by overnight shipping. For more details on where and how to ship the specimens refer to the section below on how to preserve and ship suspect EAB parasitoids from YPT samples.

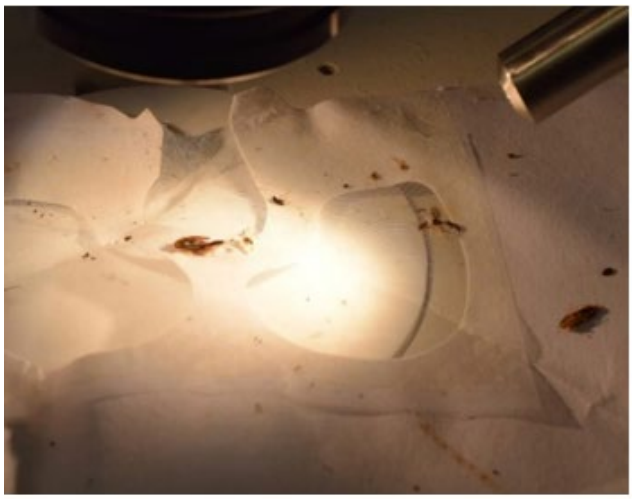
What do you need to sort through samples?

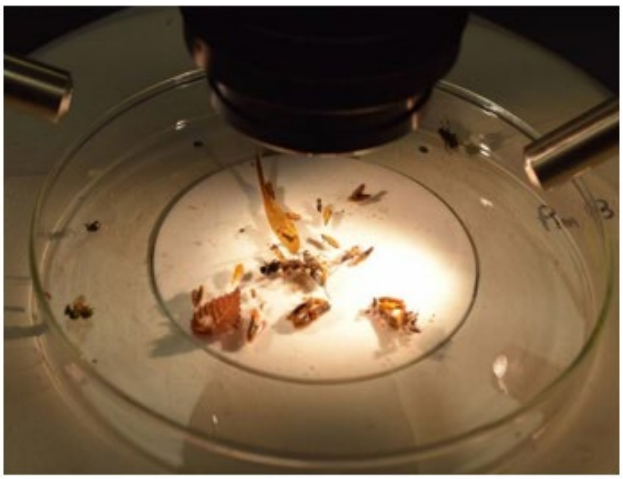
1. Dissecting microscope
2. Two pairs of fine forceps
3. A fine paintbrush 0, 00, or 000 paintbrush
4. 95% Ethanol (for and preserving specimens)
5. 70% Ethanol (for checking the samples)
6. Small screw top vials (for specimens that need confirmation ID)
7. A small dropper to help add or remove specimens from the vials (optional)
8. A glass or plastic Petri dish to hold insect samples during the sorting process

Start by taking your samples out of the freezer if necessary. If they are very icy, give them some time to thaw. Try not to refreeze samples once thawed to help preserve the insects. If you have already thawed them, you can leave the samples in the refrigerator for a day or two while you process them. Until you are very comfortable identifying the parasitoids it is a good idea to look through your reference parasitoids from the rearing facility right before processing your pan traps to familiarize yourself with the look and size of the insects you are searching for. While you may be looking at your reference parasitoids under high magnification for details, also remember to look at your reference parasitoids under the lowest magnification that you will be using as you look through your samples.

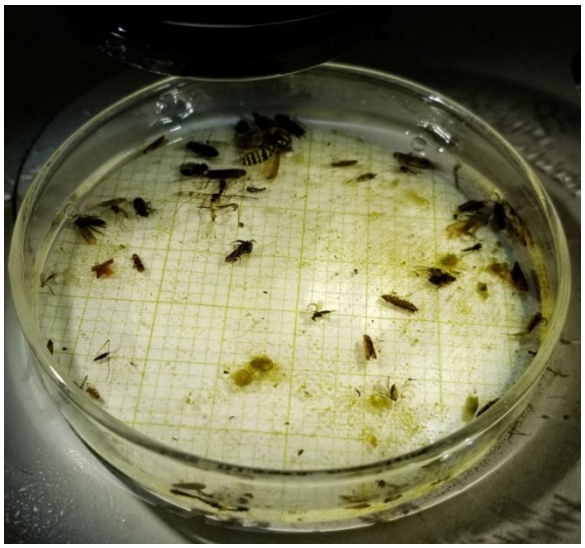
To start processing, open the zippered bag and record the location, date of the sample (including year), and pan trap number on your data sheet. Carefully unfold the filter paper, pull the filter apart at the seam, and dump the contents into your Petri dish. You may need to check the bag or outside of the filter if insects have slipped out of the filter. This may happen, especially if the filter is not properly folded over.

Systematically sort through the insects caught on the filter under the microscope at the lowest magnification, looking for anything that looks like the EAB parasitoid species. You can use your paintbrush and/or forceps to help manipulate and check the sample. If you find something, look at it under a higher magnification and refer to Appendix C the parasitoid check lists and your reference specimens from the rearing facility. If you think it may be an EAB parasitoid, please follow the instructions below under "**How to preserve and ship suspect EAB parasitoids".**

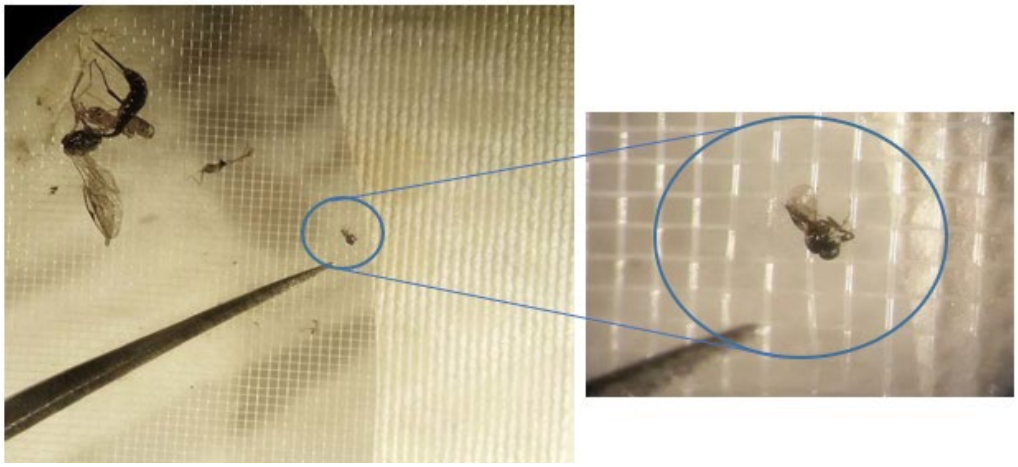




On the left a filter paper spread out and ready for viewing under the microscope. On the right a sample that has been poured onto a glass Petri dish ready for viewing under the microscope



A sample where 70% alcohol has been added to help separate the insects and where grid paper has been added to help provide reference points for sorting through the sample.



Under lower magnification the insect near the forceps may be a specimen of interest. Readjusting to higher magnification makes it clear that the body shape does not match that of *Oobius agrili*.

Once you systematically check the filter paper, check the Petri dish containing all the larger insects that you dumped out. Systematically go through the sample sweeping top to bottom and then gradually left to right until you look through the entire sample. We find it helpful to touch each insect and move it aside after determining that it is not a suspect EAB parasitoid. It may be possible for smaller insects to stick to a larger insect or to some of the leaves or debris in your sample, so be sure to flip over insects and leaves and spread-out debris to make sure you are not missing any small insects. Be sure to check the legs and tarsi of larger insects as parasitoids often become tangled and trapped there. This commonly occurs for *O. agrili*. You may find in helpful to add 70% ethanol to help break up the sample, so everything is not 'stuck' together. Some processors have also suggested adding a grid to the bottom of your dish to help you sort through the sample systematically without missing anything. Finally, make sure that you have labelled and filled out your datasheet for that sample (Appendix I), toss away your insect and filter paper, and move onto the next sample.

How to preserve and ship suspect EAB parasitoids from YPT samples

If you think you have recovered an EAB parasitoid, place it in a vial with 95% ethanol. Please us a vial with a capacity of at least 1 dram. Please do not use vials with very tiny openings since identifiers will need to insert forceps to remove the insects. Include inside the vial a paper label with all the following info written in pencil. If you label the outside of the vial in marker or pen and then it is exposed to ethanol the information will wash off:

- Mapbiocontrol Site ID# (from mapbiocontrol)
- Date
- Recovery ID # (from mapbiocontrol. This is generated after you put in a possible recovery on the site)
- Town and State

The Recovery ID# is generated when you create a recovery entry in MapBioControl. Before you send your samples, please create your recovery entries in mapbiocontrol and put the Recovery ID# on the paper in the vial with the insects. Because you can have more than one vial on a given date at a site, some vials will have the same Recovery ID#. Include the Mapbicontrol Site ID# with each sample sent for cross reference. Having this information, the insect identifier can easily enter the identification information into Mapbicontrol so that you can see if you have indeed recovered EAB parasitoids.

Once you have accumulated several specimens for identification, pour most of the ethanol off each vial, and ship them overnight to Andrea Anulewicz, UDSA APHIS PPQ, 5936 Ford Ct, Ste. 200,

Brighton, MI 48116. This alcohol must be poured off before shipping due to shipping restrictions, but alcohol will be added to the vials by the receiver.

OVERVIEW OF IDENTIFYING EAB PARASITOIDS IN YELLOW PAN TRAPS

The following provides a basic discussion of each parasitoid and a description of the identifying characteristics to look for. Please see the attached Appendix C for check lists that can help you identify each parasitoid. For a more detailed discussion of the parasitoids and how to identify them please see Module 3 Emerald Ash Borer Release and Recovery Guidelines virtual training. This training also includes a quiz that can be used to test your knowledge.

Tetrastichus planipennisi **(Eulophidae)**

Tetrastichus planipennisi female. Photo by T. Booth USDA.

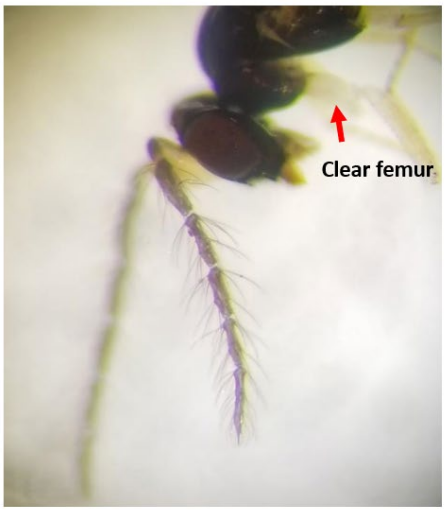
Tetrastichus planipennisi are eulophid wasps with dark bodies, red to dark red eyes and light legs with dark femurs. Females have a tapered shape (females). Do not go by size alone because they can get very small if they are from a particularly large brood.

Tetrastichus planipennisi females can be identified by 5 characteristics: 1) dark femurs, 2) 6 segment antennae, 3) abdomen that is longer than its thorax, 4) last segment of abdomen that is three-four times as long as it is wide at the base, 5) Smooth thorax with two lateral grooves on the middle thoracic segment. Note this last characteristic may be hard to see and is optional to look for. Please see Appendix C for photos.

Tetrastichus planipennisi males can be identified by 4 characteristics: 1) dark femurs, 2) 7 segment antennae with hairs that look like ‘eye lashes’, 3) Last segment of abdomen not elongated, 4) Smooth thorax with two lateral grooves on the middle thoracic segment. Note this last characteristic may be hard to see and is optional to look for. Please see Appendix C for photos.

***Tetrastichus planipennisi* Look-a-likes**

Below are two photos of insects that look superficially like Tetrastichus planipennisi with arrows indicated where they don't match the checklist or general characteristics of the species.



On the left an insect that looks like *T. planipennisi* but does not have 6 segmented antennae and the last segment of the abdomen is not four times as long as it is wide at the base. On the right the insect has clear femurs and is also not *T. planipennisi.*

***Oobius agrili* (Encyrtidae)**

Oobius agrili released for EAB biocontrol are all parthenogenic females, i.e. they do not need to mate, and they only produce daughters. Adults are very small (~1 mm long), have compact stout bodies with no waist, and a visible, short ovipositor. Their body color is black to dark brown with a blue-green sheen, and reddish eyes.

Oobius agrili can be identified by 3 characteristics: 1) Stout body, no waist, 2) Second to last antennal segment is light, all other segments are dark, 3) Clava, last segment of antennae, is

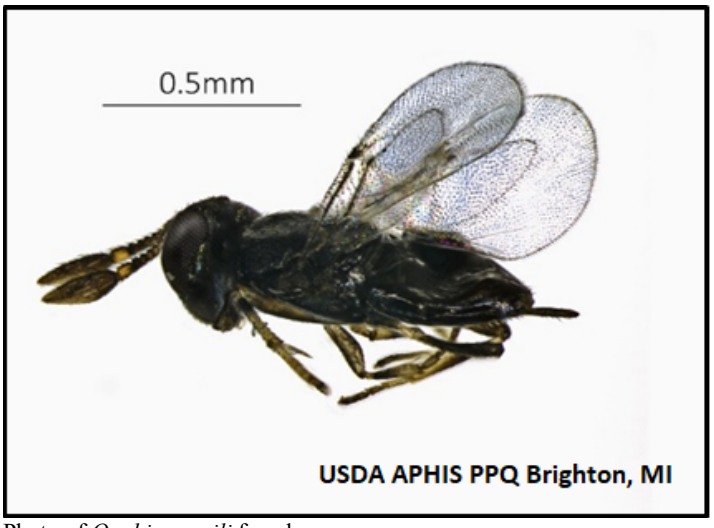


Photo of *Ooobius agrili* female.

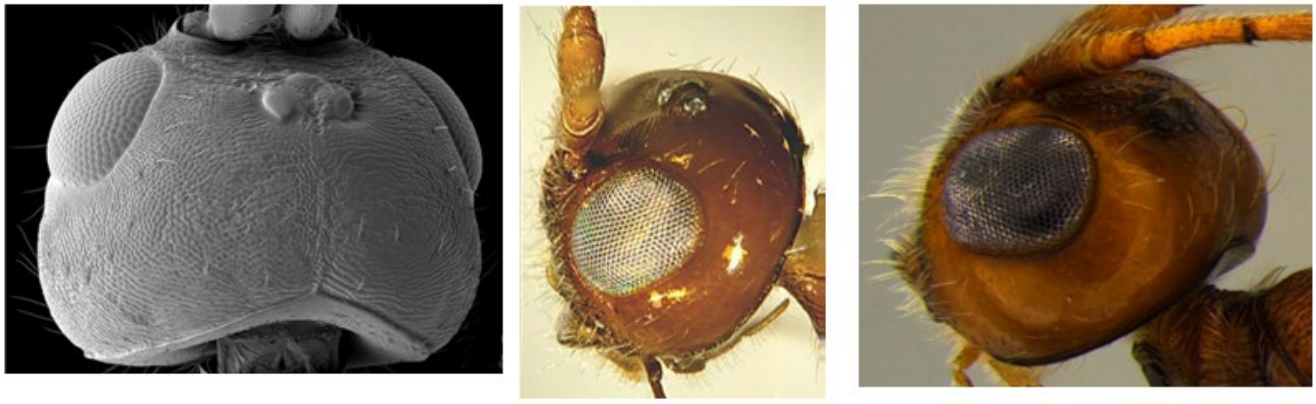
tapered upwards to a blunt point when the insect is viewed in profile.

***Spathius* species (Braconidae)**

On the left *Spathius galinae* and on the right *Spathius agrili*. Photos by T. Booth USDA.

The bodies of *Spathius agrili* and *Spathius galinae* are reddish brown except for the abdomen, which is dark brown or black. Their wings have brown veins. The forewings-tinged brown with clear banded areas. The hind wings are clear but still have brown veins. The antennae are very long, >26 segments with a total length that is 1.2 times the length of the body (without ovipositor). In the US there are also native species of *Spathius.*

One very common native is *Spathius floridanus*. *Spathius floridanus* is a deep chocolate brown over the entire body and the back of the head has wrinkles. The back of the head and cheeks of both *S. agrili* and *S. galinae* are totally smooth and wrinkle-free (see photo below).



On the left is the head of the native Spathius floridanus with lots of wrinkles, photo from Marsh and Strazanac (2009). In the middle is *Spathius agrili* and on the right is *Spathius galinae.* Both *S. agrili* and *S.galinae* have smooth cheeks and the back of their heads are smooth.

Spathius galinae can be identified by 5 to 6 characteristics. 1) Forewings-tinged brown with clear spots. Wings with brown veins, 2) Spherical head and spherical eye. 3) Back of the head smooth and

without wrinkles, 4) Base of the tibia of all legs is yellowish, 5) Pedicel, narrow waist connecting the thorax and abdomen, present and short, 5) Long antennae >26 segments. 1.2 x length of body, and 6) IF FEMALE: Long ovipositor. See Appendix C for photos.

Spathius agrili can be identified by 4 to 5 characteristics. 1) Forewings-tinged brown with clear spots. Wings with brown veins, 2) Spherical head and spherical eye. Back of the head smooth and without wrinkles, 3) Pedicel- narrow waist connecting the thorax and abdomen is present and short, 4) Long antennae >26 segments. 1.2 x length of the body, and 5) IF FEMALE: Long ovipositor. See Appendix C for photos.

***Spathius* look-a-likes**

Below are two photos of insects that look superficially like *Spathius* with arrows indicating where they don't match the checklist or general characteristics of the species.

On the left, an insect that looks like a *Spathius* but has clear wings and a yellow body. On the right, the insect looks like a *Spathius* but has short antennae, a head that is not spherical, and swollen femurs.

Native or Naturalized Parasitoids

You may come across some native or naturalized parasitoids of EAB when you are sorting through your yellow pan traps. You are not required to record or ship these parasitoids for identification, but you may find it interested to know if they are present at your site so photos of them are provided below.

Atanycolus spp.

A few species exist, all are large native parasitoids with a very distinct red or orange abdomen. In contrast, the head and thorax are jet black. The wings are dark grey.

Atanycolus sp. on an ash tree. Photo by Jian Duan, USDA-ARS.

Phasganophora sulcata

A native parasitoid solitary endoparasitoid. Distinct red/orange section on the abdomen. Thorax is black and pitted. Hind leg femur is swollen with teeth.

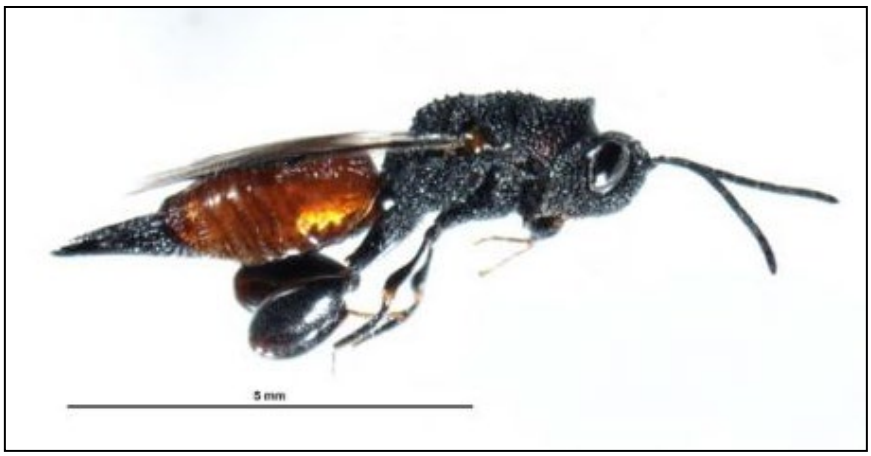


Photo of *Phasganophora sulcata.* Photo by J. Duan, USDA-ARS.

Balcha indica

naturalized in eastern U.S. Native to southeast Asia. Solitary ectoparasitoid of EAB. Pronotum textured. Dark brown with copper, blue, green, and purple lusters under different angles of light.

Photo of *Balca Indica*. Photo by J. Hansen.

TREE FELLING AND DEBARKING

Felling trees to determine parasitoid establishment can be done in the fall, winter, or early spring, at least one year after the final release at a given site. Select four trees within or near where the releases occurred that are alive (based on bark peeling and confirmation of live phloem), show signs of fresh damage due to EAB (woodpecker holes, bark splits, epicormic shoots), and are less than 10-inches DBH. At least two trees should be "small" trees under 4 inches in diameter (research has shown trees under 4.3 inches are more likely to be parasitized by *Tetrastichus plannipennisi*). Give each tree a unique ID number, and record its DBH, location (GPS coordinates), and the date the tree was felled. We generally cut these trees into 1-meter logs to make them easier to handle and peel.

Bark sampling logs for *O. agrili*

Bark samples to recover eggs can be taken from ash trees felled for other recovery work. We suggest taking one bark sample per tree from a section of the tree where there is flaky bark and there are many fresh woodpecker holes. You are more likely to find EAB eggs by sampling a part of the tree trunk that shows signs of recent EAB infestation and is still alive (i.e. the bark is still attached to the sapwood). It is not worth sampling for EAB eggs if the log shows no signs of fresh EAB infestation. From each log section, mark off a vertical area of bark 10 x 100 cm, debark this area of bark with a knife by shaving

flakes of bark onto a piece of heavy plastic or into a plastic tote and then pour each bark sample into a large paper lunch bag (reinforce the bottom with tape before sampling so bark/eggs don't fall out of the bag), label the bag with site #, tree # and date, and rear or sift the bark samples as described below. A knife is easier than a drawknife for scraping the bark of a log section because you can use one hand to hold the log and the knife to scrape the bark. Always scrap away from your body for safety. For bark sifting we recommend sampling at least 10 trees per site. So if you are felling 4 trees for larval parasitoid recovery, we recommend taking bark samples from 6 additional trees that you do not fell, to reach 10 total.

Bark sampling live trees for *O. agrili*

You can take bark samples from living, EAB-infested ash trees in the vicinity of the original release site in the field, or from trees felled for larval sampling. Fresh woodpecker feeding holes and/or live epicormics shoots in the lower half of the tree are good evidence that the tree is infested with EAB and suitable for *Oobius* bark scraping. It is recommended that trees have at least 10 fresh woodpecker holes within the first 3 to 4 meters above ground but the more the better. Five to 10 woodpecker holes at breast heigh would be an ideal tree to sample.

To sample trees in the field, mark off a vertical area of bark 10 x 100 cm on the south, southwest, or west side on the lower trunk (about 1-m above ground) on at least 10 trees. To collect the bark samples, you have two options:

Sheet method: Lightly wrap a piece of heavy plastic sheeting around the base of the tree with duct tape. Hold the edges of the sheeting up in the shape of an inverted cone (this method requires two people). Using a drawknife, shear off a thin layer of outer bark within the delineated area, and the bark debris will fall into the inverted plastic cone. Remove the duct tape, and using the plastic sheeting, funnel the bark sample into a labeled large paper lunch bag (tape the bottom of the lunch bag before using it to prevent bark/eggs from spilling out), and return it to the laboratory.

Bark sampling using the sheet method for *Oobius agrili.*

Tote method: Modify a 12-to-18-gallon tote by cutting the lip on one side of the tote and putting duct tape over the cut edge. Add extra tape around the corners to reinforce them (see attached photo). The corners are the points most likely to weaken and break from pushing against the tree and the tape helps prevent breakage. Cutting the lip allows the tote to sit flush against the tree with no gap. Cut two small holes on either side of the tote and attach a rope to the top of the tote which helps hold the tote around you while scraping (you can put the rope around your neck). Once in the field press the plastic tote into the tree so that there is no gap between the tote and the tree. Using a drawknife, shear off a thin layer of outer bark within the delineated area. Slice off multiple shallow layers of bark using a drawknife until the entire area has been debarked. Once a sample has been collected dump it into a paper bag (tape the bottom of the lunch bag before using it to prevent bark/eggs from spilling out). Brush any residual bark from the tote into the paper bag. Label the sample with the date, site, state, tree number, and DBH.

Bark sampling using the tote method for *Oobius agrili*

Rearing *O. agrili* from bark

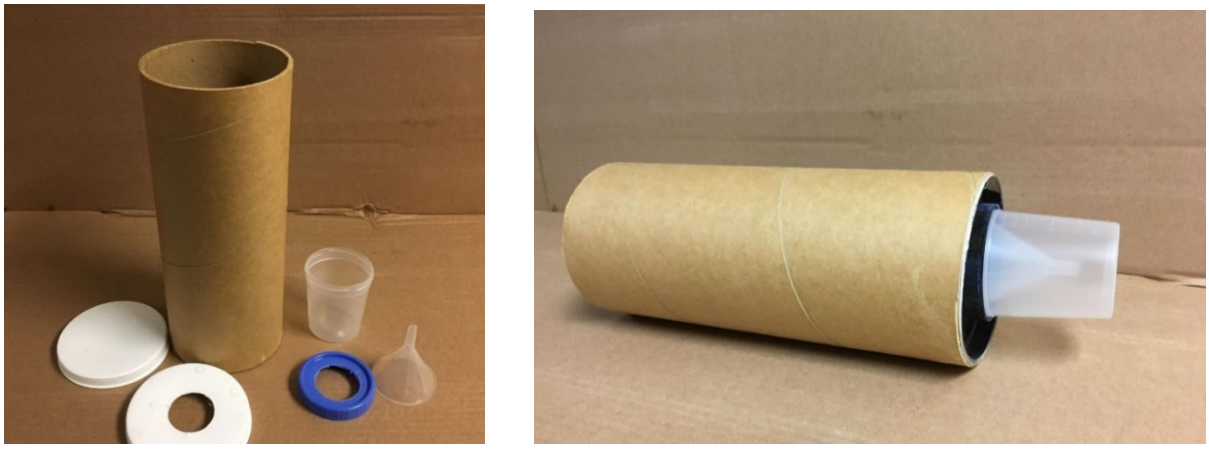
Oobius agrili overwinters inside EAB eggs as mature larvae. They emerge from eggs in the spring around 650 GDD50F. Bark samples can be reared in small rearing containers to collect adult *O. agrili* that may be overwintering in EAB eggs. These rearing containers can be constructed from 4" diameter cardboard poster tubes with tight-fitting plastic plugs. Only a small number of parasitized EAB eggs will contain overwintering *O. agrili* that haven't yet emerged, so a larger number of bark samples should be collected if you do not plan to sift the bark after rearing. If only rearing bark samples, we recommend taking 30 bark samples per site. If trees are limited, more than one bark sample may be taken from larger trees. If you choose not to rear the bark, then proceed to "Sifting *O. agrili* from bark."

- **Supplies needed for rearing *O. agrili* from bark**

1. 4" diameter poster tubes cut 10-12" long and with tight fitting plastic plugs for each end.
2. Black spray paint designed to adhere to plastic.
3. 4.5 oz. clear specimen cup with lid.
4. Small clear funnel. Large end 2" and funnel end 0.25" in diameter.
5. Hot glue gun.
6. 1.5" diameter hole saw bit for drill. Alternatively, a sharp knife and extreme care

- **Construction and collection from bark rearing tubes**

The outer surface of plugs should be painted black with spray paint designed for application onto plastic. This will reduce light transmitted through the plugs and direct emerging adult parasitoids to 1.5–inch opening you will cut in one of the plugs. Cut a 1.5"-diameter hole in the center of one of the plugs. Next, cut a 1.5" diameter hole in the cap of a 4.5-oz. specimen cup. Glue the larger end of the funnel to the bottom side of the specimen cup lid (side with threads) using hot glue. Then glue the top of the specimen cup lid to the outside surface of the poster tube plug, centering the holes that were drilled. Make sure that there are no tiny gaps between the plug surface and cap surface that would allow parasitoids to escape. The specimen cup can then be screwed onto the cap. Apply a streak of honey to the inside of the specimen cup to provide food for any emerging parasitoids. Check cups at least every other day for adult emergence. If parasitoids are present, you can squirt them with ethanol to prevent them from escaping and use a small brush to place them in vials with ethanol. Hold bark in rearing containers for at least 6 weeks at room temperature to allow all parasitoids to emerge.



On the left are the parts needed for construction of rearing tubes for rearing *O. agrili* from bark. On the right is a completed rearing tube for rearing *O. agrili* from bark.

Sifting *O. agrili* from bark samples

Dry the sample at least one month at room temperature (if the bark was already processed for rearing *O. agrili* it will already be dry), place small aliquots of bark in a No. 14 covered soil sieve and shake vigorously for several minutes to sieve EAB eggs, small insects and other small debris from the bark sample into a white ceramic baking pan. Using a dissecting microscope to sort through each bark sample, we recommend following the method described by the Minnesota Department of Agriculture. Their presentation which includes many photos of parasitized EAB eggs and look-alikes can be found at: Biological Control of Emerald Ash Borer: Bark Sifting for Oobius agrili.


On the left a No. 14 covered soil sieve for sorting bark. On the right a white ceramic baking pan with sifted bark ready for viewing under a dissecting microscope.

If you find EAB eggs or parasitoids

Collect all EAB eggs and small adult parasitoids into a small Petri dish with a friction-fitted lid (Fisher Scientific 50 X 9-mm dishes – catalog number 08-757-105) labeled with state, site ID #, recovery ID # and date of collection (DD-MMM-YY, ex. 05-Feb-24) using a fine-tip permanent marker. Ship the EAB eggs and parasitoids collected from bark sifting or reared from bark or logs to Toby Petrice, Forest Service, 2601 Coolidge Rd, Ste. 203, East Lansing, MI 48823.

Peeling logs to recover larval parasitoids

The USDA has a short 5-minute video called "Debarking Ash Tree Logs to Look for Emerald Ash Borer" available on YouTube at: https://www.youtube.com/watch?v=sMV-1r5lnvs&t=9s

Both species of larval parasitoid, *Tetrastichus* and *Spathius*, can be found in EAB galleries under the bark. We recommend felling trees for debarking between November and March. Below is a guide to peeling ash logs to look for EAB, please reach out if you need clarification.

An ash log with the bark peeled back revealing EAB galleries.

1. **Peeling the ash logs**

- Logs are easiest to peel if debarked soon after felling.
- If you need to store the ash logs, try to store them in a way that reduces moisture loss. You can keep them in a cold chamber and/or store them in a barrel of water or seal the ends (with Anchorseal® for example) to reduce moisture loss.
- It is easiest to peel using a large 9-15" drawknife.
- If the bark is too thick, you can remove the outer bark with a draw knife first. If you find an EAB gallery, remove the phloem in that specific area, be careful with your drawknife or for more precision use a sharp chisel or small 5" drawknife.

- The phloem will easily separate from the outer sapwood when the ash logs are fairly fresh. Phloem and layers of outer sapwood will be harder to remove in areas where the tree is dead and dried out but EAB and larval parasitoids can still be present in these areas if the tree died in the current year.

2. Inspecting all EAB galleries

Examine the end of each EAB gallery for an EAB larva or signs of parasitized larvae. You may see the follow fates.

a. EAB larva parasitized by *Tetrastichus* may: (see Appendix B for photos)

1. look healthy*
2. appear lumpy like a "braided rope"
3. be replaced by a mass of small grub-like larvae (white), pupae (color ranges white to bluish-black) and/or adults (dark metallic blue)
4. have emerged from the EAB gallery, leaving only the head and tail of the EAB larva and small black spots in the gallery called meconium (the spots are waste excreted by each *Tetrastichus* larva before pupation is complete). The head and tail may not always be visible.

**Tetrastichus* larvae remain inside the EAB larvae for at least a week before emerging from the EAB. If you have access to a laboratory, you may find more parasitized larvae if you place 3rd, 4th, and J-larvae in a petri dish and dissect it. Many small eggs and parasitoid larvae can be seen swimming in the EAB haemolymph (See appendix B for helpful dissection photos). Note: EAB larvae can be saved in labeled petri dishes, stored in the fridge, and dissected weeks later.

b. EAB larva parasitized by *Spathius:* (see Appendix B for photos)

All life stages live on the outside of the host. *Spathius* eggs and small larvae are difficult to see with the naked eye. But by late fall, most will be large larvae or will have spun silken cocoons that are fairly easy to see in the EAB galleries.

c. EAB larva parasitized by a single solitary larva or cocoon

An EAB parasitized by a single larva or cocoon is probably *Atanycolus* (native parasitoid), or *Balcha indica* (naturalized parasitoid). You do not need to ship these parasitoids. (see Appendix B for photos)

d. A healthy EAB larva

If you find a health EAB larva that is a small instar, 1st or 2nd, it does not need to be dissected. If you find a healthy EAB larva it is a larger 3rd, 4th or J larvae you can dissect it for signs of internal parasitism by *Tetrastichus*. A J larva is not going to be visible until you chisel into the wood of the tree (see Appendix B for photos).

e. You may find an empty gallery where a woodpecker has taken the larva.

You can use a sharpie to marker woodpecker holes before peeling so you know why the gallery you've peeled is empty. Recording data on woodpeckered EAB is not required. However, some forest managers may opt to record this data for a more comprehensive understanding of the impact of woodpeckers within their forest stand or for research purposes.

f. A dead EAB larva

EAB can die for a variety of reasons including tree defenses, fungi, viruses, or extreme cold.

3. How to extract and ship suspected parasitoids

- If you find a potentially parasitized EAB/ parasitoid clutch carefully remove the EAB larva along with any parasitoid eggs, larvae and/or cocoons or pupae, and place them in a small petri dish with a friction-fitting lid (Fisher Scientific 50 X 9-mm dishes – catalog number 08-757-105 is a good choice) or a vial with a capacity of at least 1 dram. Please do not use vials with very tiny openings since identifiers will need to insert forceps to remove the insects.
- Please save each clutch of parasitoids/each parasitized EAB larvae in a separate vial or petri dish.
- Using a pencil, label a small piece of paper with the state, recovery ID#, site ID #, tree number, and date (Please use Day-Month-Year in format: DD-MMM-YYYY; ex.10-Jul-2023) and place in each vial or petri dish. Pencil is preferred as pen and sharpie can be washed away accidentally by ethanol. When you enter the data into mapbiocontrol (see below) a unique recovery ID # will be generated. This number will be used by the identifier to enter the parasitoid ID into mapbiocontrol.org.
- Ship the specimens within 48 hours to Andrea Anulewicz, USDA APHIS PPQ, 5936 Ford Ct, Ste. 200, Brighton, MI 48116 for identification. If you need to keep samples longer before shipment add 95% ethanol to preserve the specimens and then remove most of the ethanol before shipment.

4. **Enter the data into Mapbicontrol.**
   - Peeling 4 trees at one release site will count as one recovery entry. The identifiers will enter the final identification data of *Tetrastichus or Spathius* into Mapbicontrol.
   - Please record your peeling event and if you have found potential parasitoids. Please make a record on Mapbiocontrol even if you do not find parasitoids. If you found parasitoids, please note in the comments how many potentially parasitized clutches you are shipping to Andrea Anulewicz, USDA APHIS PPQ, 5936 Ford Ct, Ste. 200, Brighton, MI 48116.
     ***Note count the number of EAB larvae that were parasitized not the number of parasitoids in each gallery. Each group of parasitoids in a single EAB gallery counts as a clutch. Please ship each clutch in a separate vial or petri dish.**
   - Once you enter the data into Mapbicontrol it will generate a Recovery ID #. Please include that recovery ID # on shipments you send to help the identifiers with matching your shipment to its online record.
   - An optional datasheet provided below in Appendix H may be used if you wish to record additional information about your EAB population and parasitoid populations as peel but is not necessary. Save & ship each brood for identification that has *Spathius* larvae, cocoons, and/or adults, *Tetrastichus* larvae and/or adults, and any clutch with gregarious(multiple) larvae or cocoons where you are unsure of species. You may also wish to take photos of each parasitoid clutch and or any suspect signs of *Tetrastichus* meconium and email those photos to Andrea Anulewicz at andrea.anulewicz@usda.gov.

For questions or concerns about tree peeling including some additional visual resources please feel free to contact Theresa Booth (*Murphy*), Biological Science Technician at Theresa.c.murphy@USDA.gov

ENTER RECOVERY DATA

The EAB Biological Control Program must have data on where EAB parasitoids are establishing. Please note entering negatives (recovery efforts where no EAB parasitoid were recovered) is also important so we have data on where parasitoids are not establishing. Once you have completed surveys to detect established parasitoids, enter the data directly at www.mapbiocontrol.org or using the MapBio iPhone/iPad app. When you enter the mapbiocontrol.org website, click on RECOVERY in the green banner at the top. Click the New button to enter new data. You will be asked to enter the following data:

Location

- **Site ID** Enter the Mapbicontrol Site ID for your site. If you find parasitoids not connected with any particular release or control site, simply leave this blank.
- Trap ID This field is not necessary, but if you wish to record which trap collected the insects for your information, you can enter the trap number here.
- ***Latitude** (dd.ddddd)- You can just enter the LAT/LONG for Site ID if you don't have a LAT/LONG for your traps.
- ***Longitude** (dd.dddd)- You can just enter the LAT/LONG for Site ID if you don't have a LAT/LONG for your traps.
- ***Country**
- ***State**

Sampling

- **Date sampled** in format DD-MMM-YY, Ex. 20-JUN-23
- ***Sampling Method**- (Yellow Pan Traps, Tree Debarking, Logs in Tubes, Bark in Tubes, Sentinel Eggs, Sentinel Larvae, Egg Collection, Bark Sifting, Other)
- **Number of Samples**- If 'Yellow Pan Traps' enter the number of yellow pan traps you sampled that week at that site, usually 15. If 'Tree Debarking', 'Logs in Tubes', 'Bark in Tubes', 'Egg Collection', or 'Bark Sifting' enter the number of trees you sampled.
- **Date Sample Shipped**: in format DD-MMM-YY, Ex. 05-FEB-24. Left blank if no parasitoids recovered.
- **Samples Shipped to**: Who you shipped your suspect parasitoid samples to. This will most commonly be Andrea Anulewicz, USDA APHIS PPQ, 5936 Ford Ct, Ste. 200, Brighton, MI 48116. Left blank if no parasitoids recovered.
- ***Possible EAB Parasitoids Recovered?** Yes or No
- **Comments:** It is very helpful if you write which parasitoids you thought you recovered in this field. For example, you could write "suspect 2 T. planipennisi and 3 O. agrili". This will help the identifier determine what they are looking for in the vial.

***Indicates required information.**

The actual number of released parasitoids or the number of clutches of parasitoids recovered will be entered into the database by the identifiers but you can write what you suspect in the comments. If you want information on the Trap # that recovered parasitoids include that on the label you ship out.

NOTE: Once you make a recovery entry Mapbiocontrol will give that entry a Recovery ID number, it

is the first number of the Parasitoids recovery page, under “ID” Please include this recovery ID in any samples you ship so that we can match it up to the appropriate record.

Forest Type

On mapbiocontrol.org in the Release section, there is a tab for Forest Type. Collecting this data is not required. But if you have the time and resources, it will greatly assist researchers in determining which types of forest compositions are more likely to promote the establishment of EAB parasitoids.

Ash Health Assessment

On mapbiocontrol.org in the Release section, there is a tab for Ash Health Assessment. Collecting Ash Health Assessment data is not required, but if you have the time and resources, it can assist researchers in determining the trajectory of ash mortality and how it correlates with the establishment of EAB parasitoids.

EAB Life-Stages

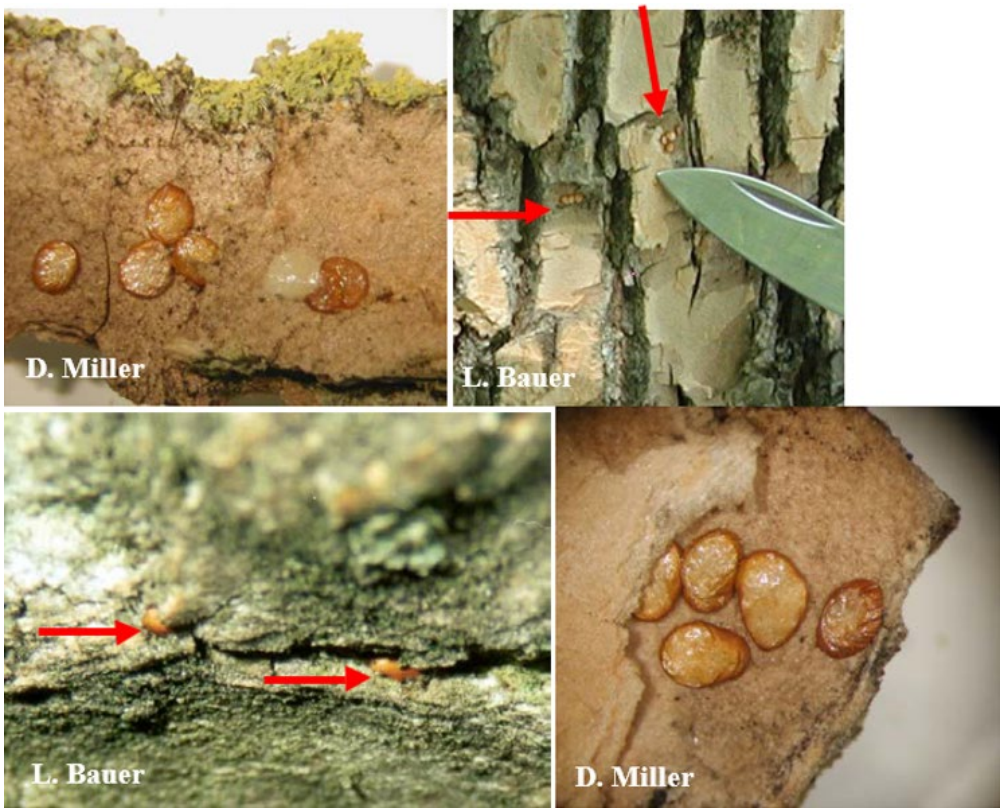


Figure A1. EAB eggs. (Top left) EAB eggs showing the newly laid egg is white, the other eggs have matured to an amber color. (Top right) clutches of eggs laid between bark flakes that have been exposed with a knife. Arrows indicate the eggs. (Bottom left) single eggs in bark crevices. Arrows indicate the eggs. (Bottom right) a close-up photo of a clutch of EAB eggs on a bark flake.

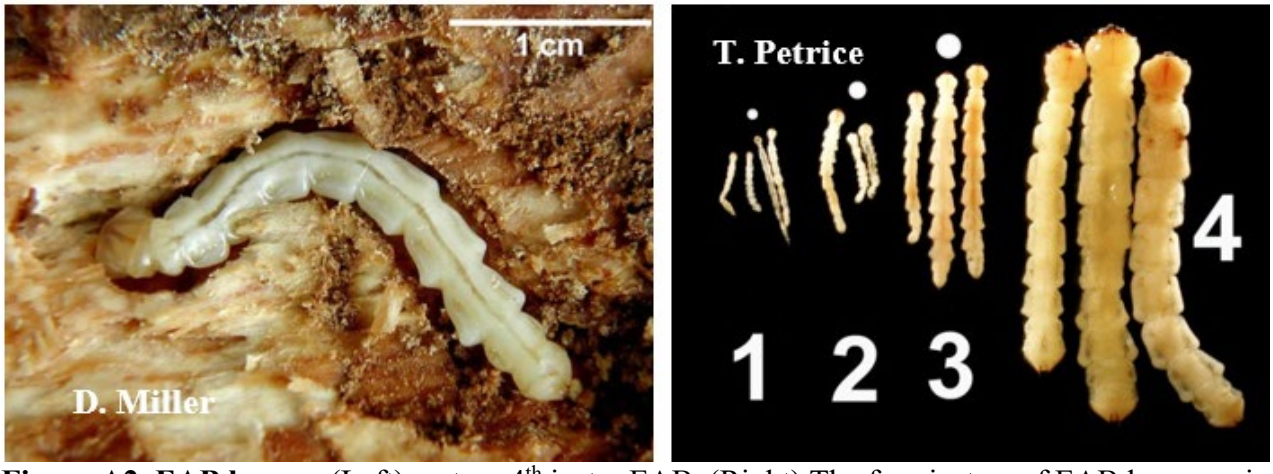


Figure A2. EAB larvae. (Left) mature 4th instar EAB. (Right) The four instars of EAB larvae going from left to right.

Appendix A – EAB life stages and damage

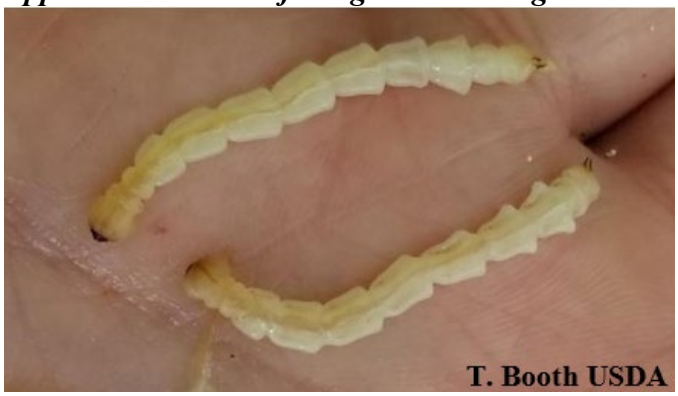


Figure A3. EAB larvae. Two 4th Instar EAB larvae. Larvae are dorso-ventrally flattened, have 10 bell-shaped segments, and a posterior pair of small brown structures called urogomphi.

Figure A4. EAB adults. Two photos of EAB adults standing on leaves.

Figure A5. Overwintering EAB Larvae. (Left) J-shaped larvae in pupal chamber in outer sapwood. (Right) EAB pre-pupa in outer sapwood.

Figure A6. Overwintering EAB Larvae. (Left) Stages of maturing pupae from left to right. (Right) EAB pupae in outer sapwood.

Figure A7. EAB larval galleries. (Left) S-shaped galleries visible on a tree that has it's bark peeled off. (Center) S-shaped gallery of an EAB larva. The larvae traveled from left to right as it fed and then double back left once it was a 4th instar. (Right) S-shaped galleries of an EAB larvae. The larvae travelled from top to bottom as they fed.

Figure A8. External signs of EAB overwintering chamber under the bark. (Left) the EAB gallery is filled with light-colored frass that indicates an overwintering EAB larvae is underneath in the sapwood. (Right) The bark has been peeled back to shows the exit portals of three pupal chambers, each with 2 holes filled with frass. Arrows indicate the portals.

Signs of EAB infestation

Figure A9. Thinning Ash Crowns. On the left and right two photos showing thinning ash crowns due to stress from EAB infestation.

Figure A10. Epicormic Shoots. (Left) Epicormic Shoots on an ash tree in the winter with no leaves present. (Right) Epicormic Shoots on an ash tree in the summer with leaves present.

Figure A11. Bark Splits with Larval Galleries Beneath the Bark. (Left) Bark split with larva gallery visible beneath the bark. (Right) Bark split after bark is peeled off tree. Not callusing around the old gallery.

Figure A12. Woodpecker Feeding. (Left) woodpecker feeding showing flaked bark and a hole where an EAB larva was removed. (Center) woodpecker feeding in the mid-crown of two ash trees. (Right) woodpecker feeding on EAB showing flaking bark and several holes in lower ash trunk.

Figure A13. Two EAB adults emerging from D-shaped exit holes.

Figure A14. D-shaped exit holes. (Left) A D-shaped exit hole with an EAB adult above it. (Right) Two D-shaped exit holes.

Additional photos and specific morphological and physiological information can be found in the EAB Program Manual and in Module 1 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 7, 2022 - YouTube An introduction and background and lifecycle of EAB, how to identify EAB and possible look-a-likes, history of biological control of EAB, how to identify ash and possible look-a-likes, impacts of EAB parasitoids and quiz on tree species and insect id. (1 hour 40 minutes).

Life stages of EAB Parasitoids

Spathius agrili

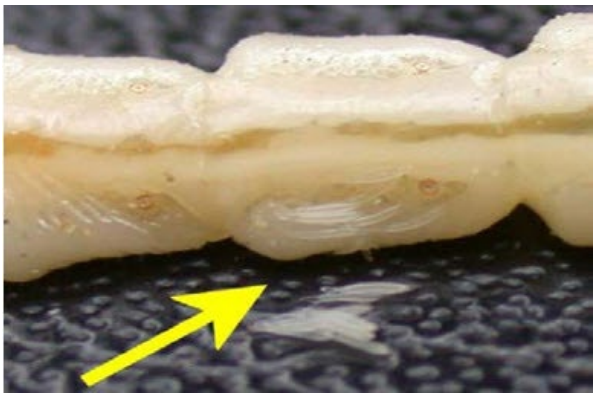


Figure B1. *Spathius agrili* eggs, indicated by an arrow, laid on the surface of EAB larvae.

Figure B2. Larvae of *S. agrili* feeding externally on an EAB larva.

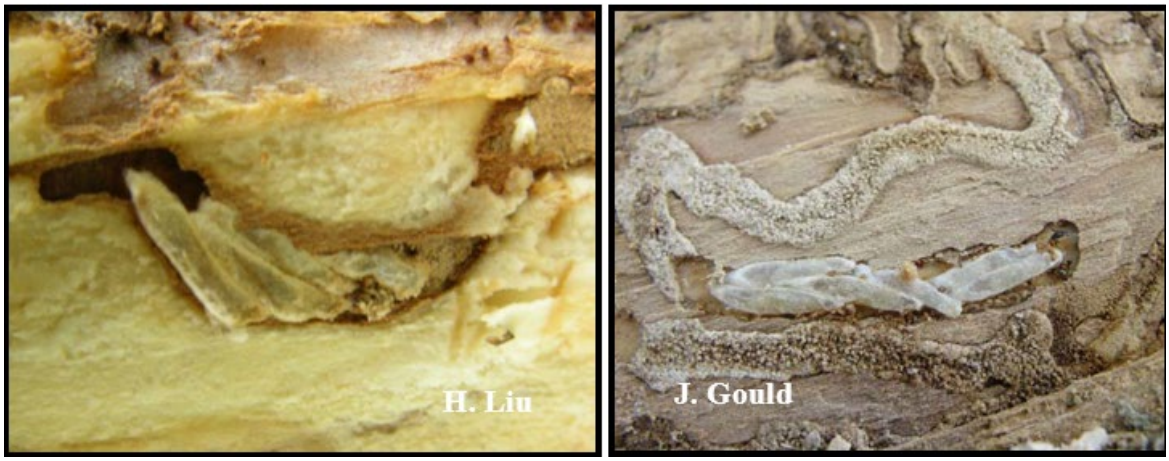


Figure B3. Two photos of silken cocoons of *S. agrili* in the host gallery of EAB. The cocoons contain mature larvae or pupae of *S. agrili.*

Figure B4. Female *S. agrili* on an ash tree. Females lay eggs through ash bark onto an EAB larva.

Spathius galinae

Figure B5. *Spathius galinae* cocoons in one gallery at the top of the photo and *S. galinae* larvae in another gallery at the bottom of the phot.

Figure B6. *Spathius galinae* adult female.

Tetrastichus planipennisi

Figure B7. Immature *T. planipennisi* larvae inside an EAB larva.

Figure B8. Mature *T. planipennisi* larvae inside an EAB larva

Figure B9. *Tetrastichus planipennisi* larvae emerged from the host. These larvae remain and pupate in the EAB gallery.

Figure B10. *Tetrastichus planipennisi* larvae and pupae in an EAB gallery. Larvae of *T. planipennisi* develop asynchronously, and larvae and pupae are often found together inside one EAB gallery.

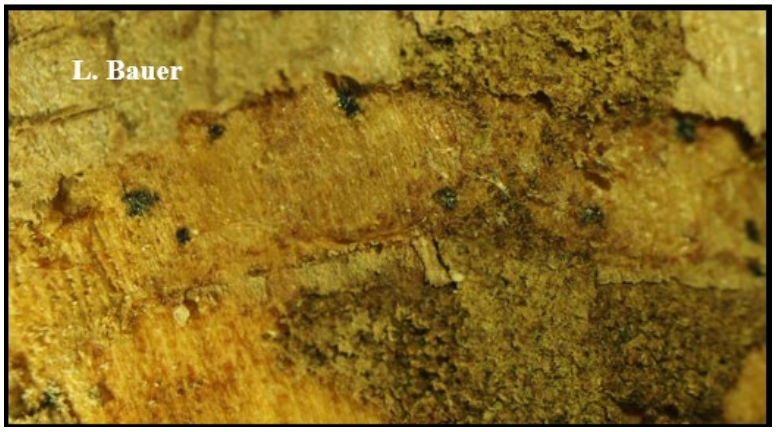


Figure B11. *Tetrastichus planipennisi* meconia (waste) in an EAB gallery. The meconia appears as black spots in the empty EAB gallery after adult emergence is complete.

Figure B12. *T. planipennisi* female lays eggs in an EAB larva through ash tree bark.

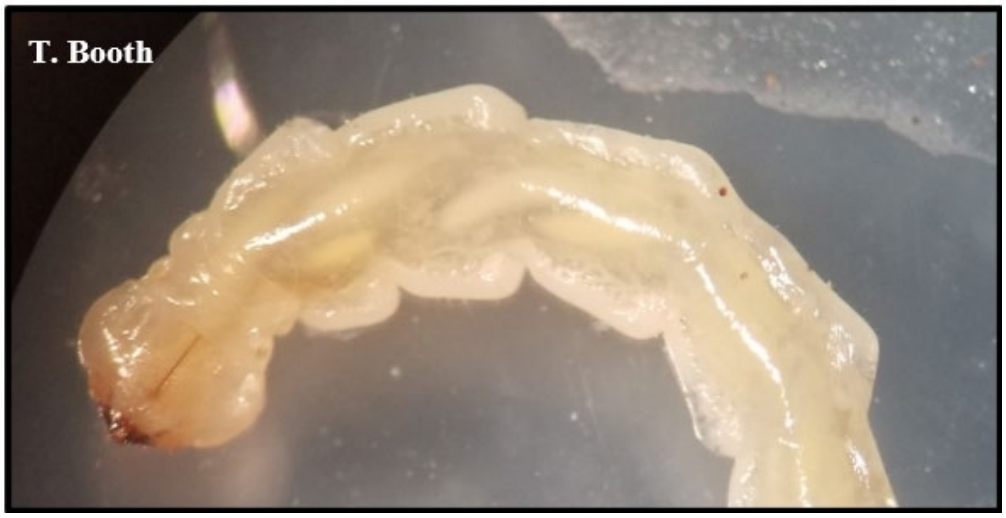


Figure B13. EAB larva ready for dissection under the scope, *T. planipennisi* larvae are visible swimming with the EAB haemolymph.

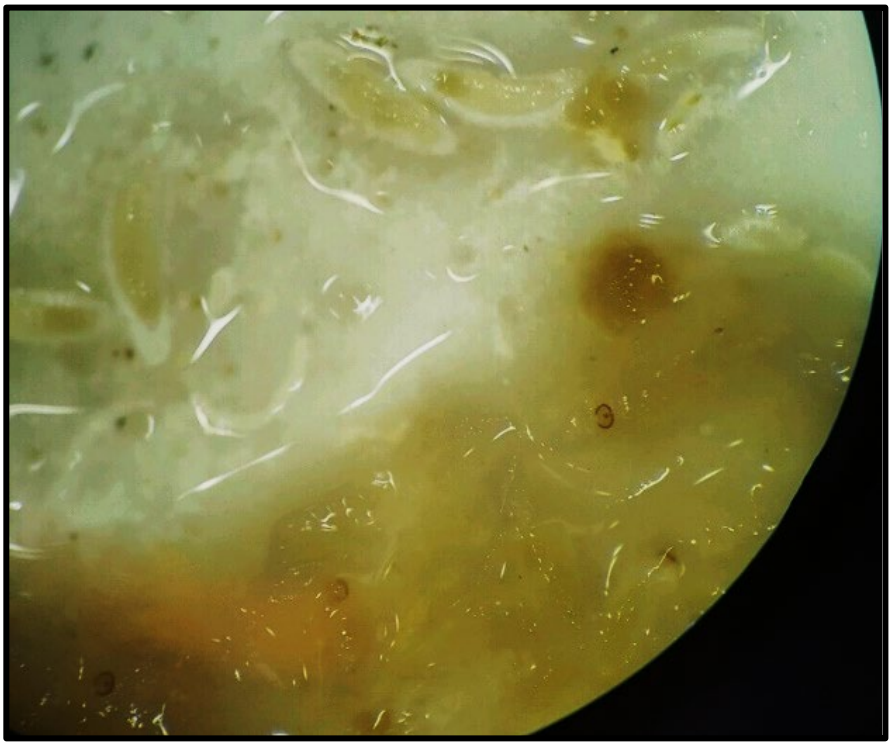


Figure B14. Dissected EAB with visible *T. planipennisi* larvae under the scope. Dissected EAB larvae may have bits and particles that look like a larvae or egg but unless you see several distinctive eggs or larvae it is not *T. planipennisi*

Oobius agrili

Figure B15. EAB eggs often turn dark brown (left egg) and then black (right egg) when parasitized by *O.agrili*; fresh EAB eggs will start off white (center egg) and turn amber in color as it develops if not parasitized by *O.agrili*.

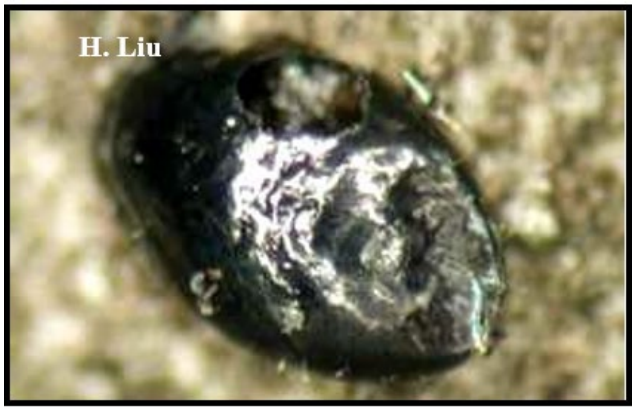


Figure B16. An EAB egg with an *O. agrili* exit hole. Adult *O. agrili* chew a circular hole through the EAB eggshell and emerge.

Figure B17. *Oobius agrili* female parasitizing an EAB egg laid on ash bark.

A few species of *Atanycolus* exist, all are native solitary parasitoids. The figure below shows the lifecycle of *Atanycolus cappaerti*.

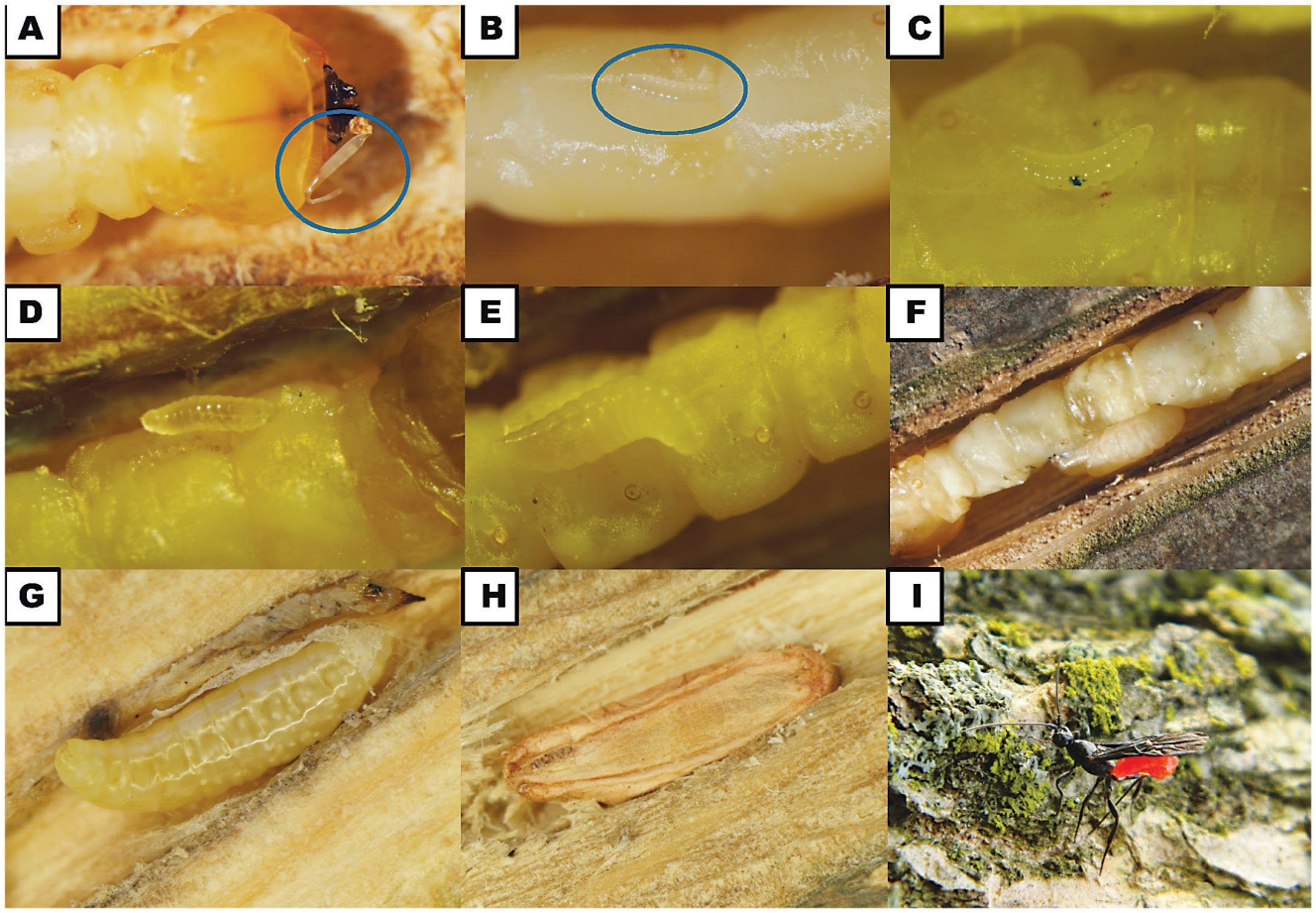


Figure B18. Stages of *Atanycolus cappaerti* figure captions. (A) Egg on *Agrilus planipennis* larva at 3× magnification; (B) 1st instar at 3× magnification; (C) 2nd instar at 3× magnification; (D) 3rd instar at 3× magnification; (E) 4th instar at 3× magnification; (F) 5th instar at 1.8× magnification; (G) 6th instar at 1.8× magnification; (H) pupal cocoon at 1.8× magnification; (I) Eclosed adult. From Duan, J. J., & Schmude, J. (2016). Biology and life history of Atanycolus cappaerti (Hymenoptera: Braconidae), a North American larval parasitoid attacking the invasive emerald ash borer (Coleoptera: Buprestidae). *Florida Entomologist*, *99*(4), 722-729. https://doi.org/10.1007/s10526-011-9408-0

Appendix B - Parasitoid life stages
Balcha indica

This species is native to southeast Asia but has naturalized in the eastern US. It is a solitary ectoparasitoid of EAB. The figure below shows the lifecycle of *Balcha indica.*

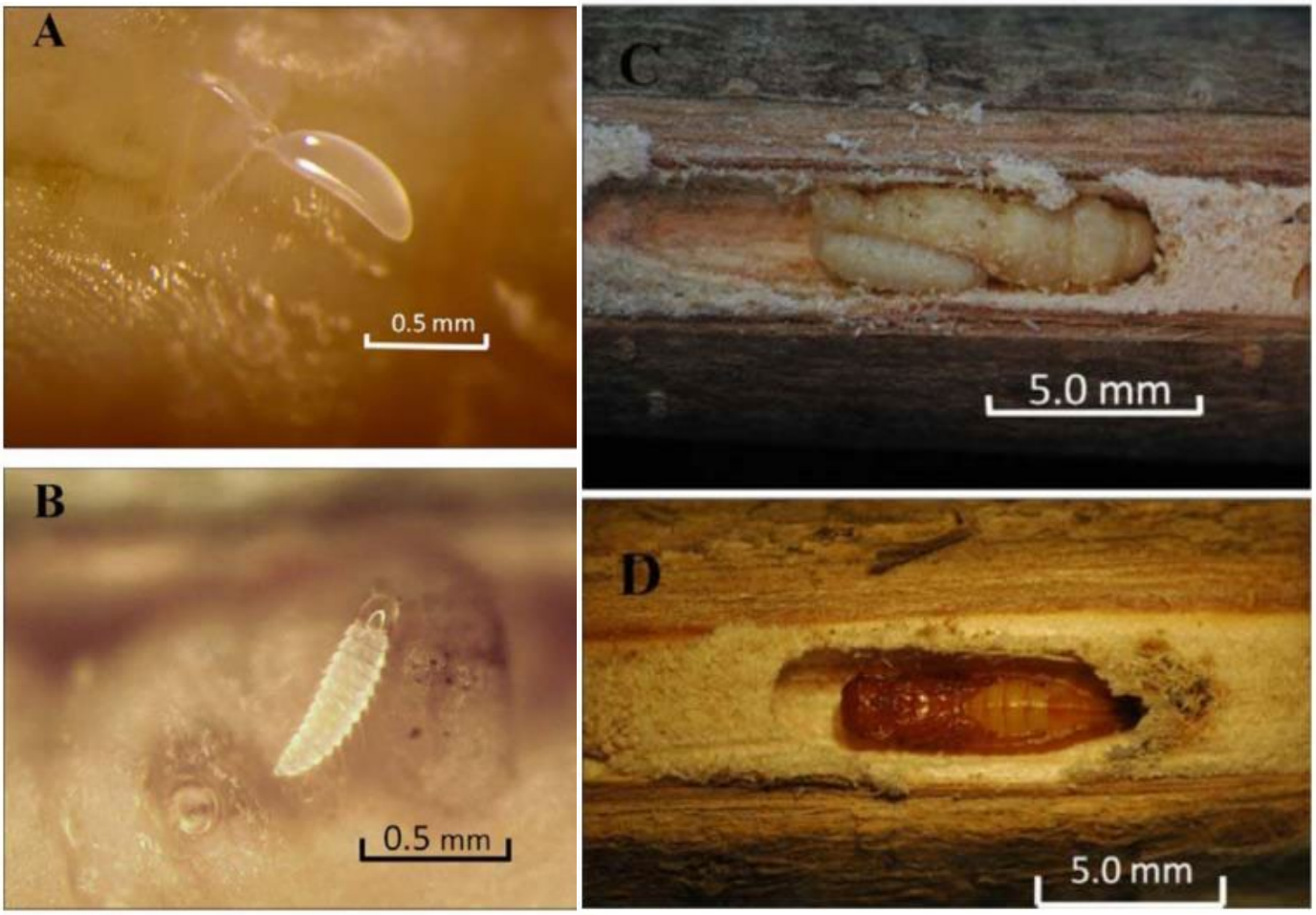


Figure B19. Stages of *Balcha indica* figure captions. (A) a single egg laid on the surface of an EAB host; (B) 1st instar larva feeding on the EAB pre-pupae; (C) the "maggot-like" intermediate-stage larva parasitizing EAB pupa; (D) pupa in cell.. From Duan, J. J., Taylor, P. B., & Fuester, R. W. (2011). Biology and life history of *Balcha indica*, an ectoparasitoid attacking the emerald ash borer, *Agrilus planipennis*, in North America. *Journal of insect science (Online)*, *11*, 127. https://doi:10.1673/031.011.12701

Phasgonophora sulcata

A native parasitoid, *phasgonophora sulcata,* dissected from an EAB larva. This parasitoid is a solitary endoparasitoid, you'll see only one larva (rarely two but one is usually dead). The larva is usually found near the tail end of the EAB. *Phasgonophora sulcata* has a distinctive head end and tail end, unlike a *T. planipennisi* larva.

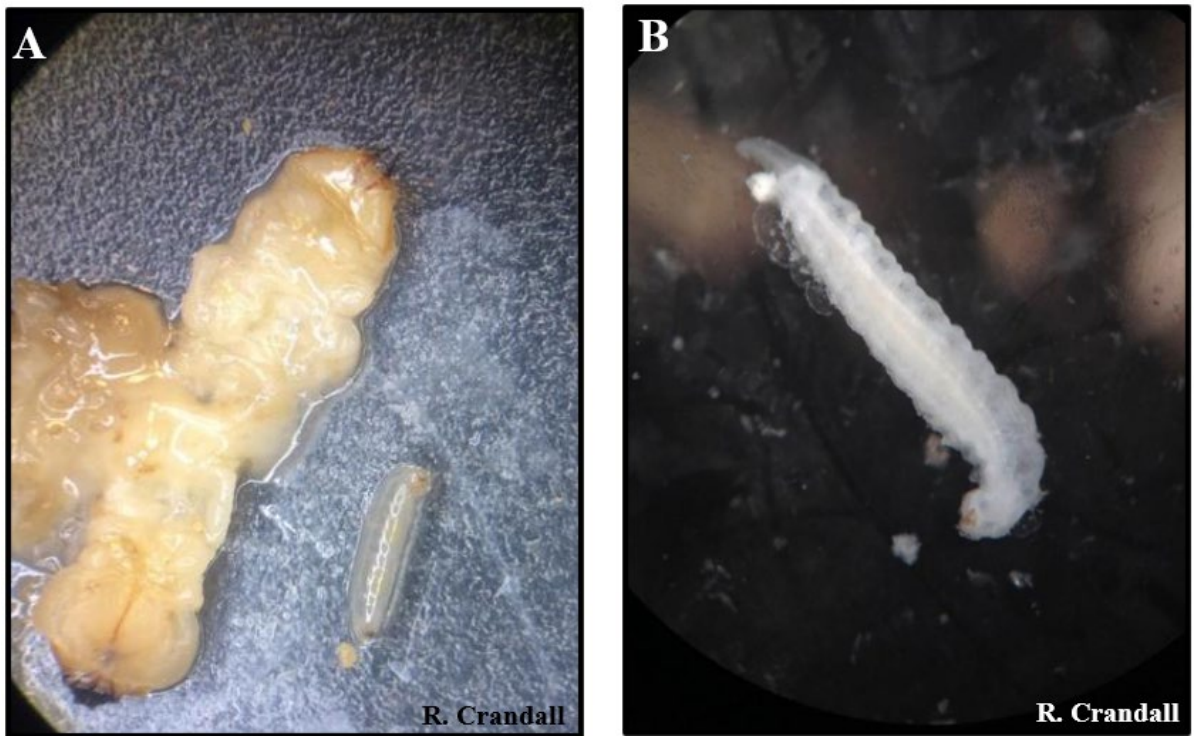


Figure B20. (A) A dissected EAB larva on the left and a *phasgonophora sulcata* on the right and (B) a phasgonophora sulcata larva with the tail at the top and the head at the bottom.

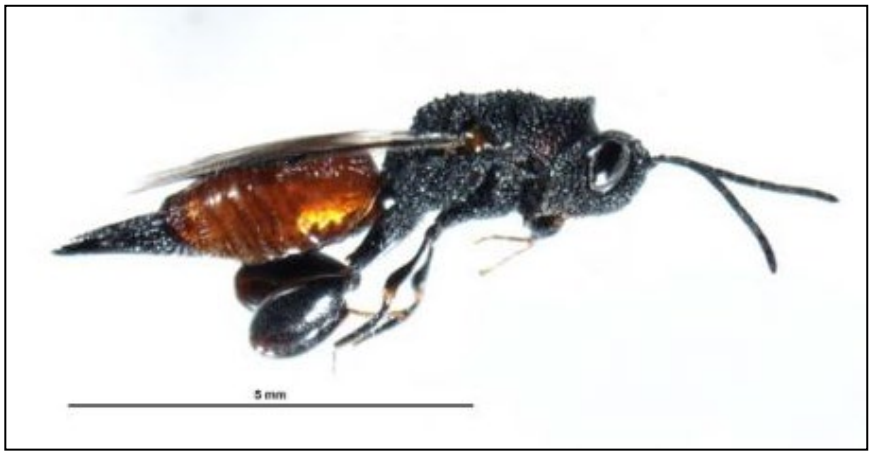
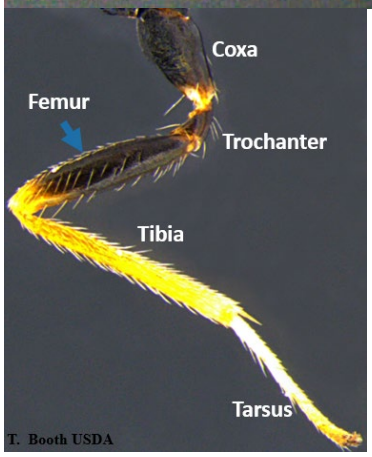


Figure 21. Photo of an adult *Phasganophora sulcata*.

Tetrastichus planipennisi female checklist

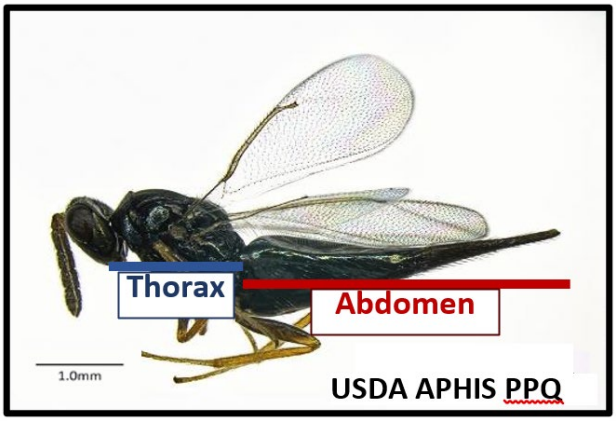
Tetrastichus planipennisi females can be identified by 5 characteristics: 1) dark femurs, 2) 6 segmented antennae, 3) abdomen that is longer than the thorax, 4) last segment of abdomen that is four times as long as it is wide at the base, 5) Smooth thorax with two lateral grooves on the middle thoracic segment. Depending on condition of your specimen some characteristics may be hard to see or confirm, you can ship any suspect specimen you are unsure about for confirmation.

1. Dark femurs.



2. 6-segment antennae: scape (1), pedicel (2), three funicles (3-5) and clava (6).

3. Abdomen longer than thorax.



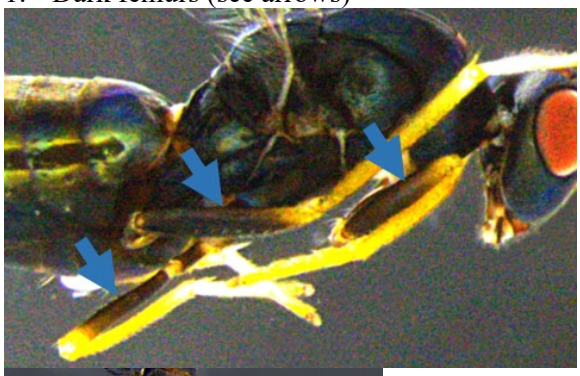
4. Last segment of abdomen four times as long as it is wide at base.

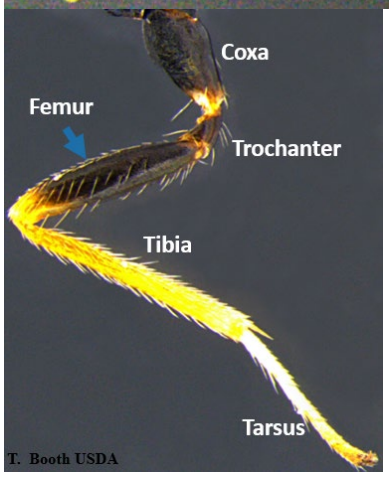
5. Smooth thorax two lateral grooves on the middle thoracic segment; optional as they may be hard to see.

Tetrastichus planipennisi male checklist

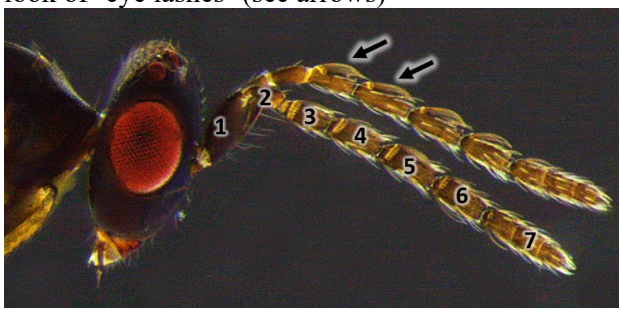
Tetrastichus planipennisi males can be identified by 4 characteristics: 1) dark femurs, 2) 7 segment antennae with hairs that look like 'eye lashes', 3) Last segment of abdomen not elongated, 4) Smooth thorax with two lateral grooves on the middle thoracic segment. Depending on condition of your specimen some characteristics may be hard to see or confirm, you can ship any suspect specimen you are unsure about for confirmation.

1. Dark femurs (see arrows)

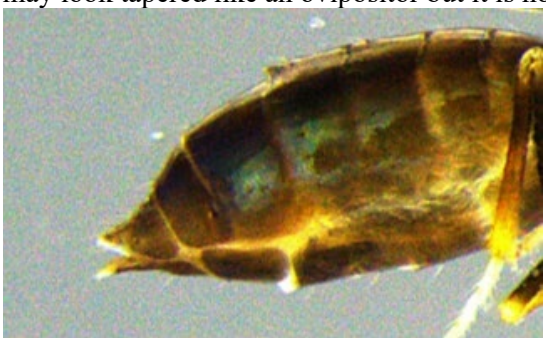




2. 7 segment antennae : scape (1), pedicel (2), four funicles (3-6) and clava (7); some hairs having the look of 'eye lashes' (see arrows)



3. Last segment of abdomen not elongated. The aedeagus may be visible and/or the end may look tapered like an ovipositor but it is not.

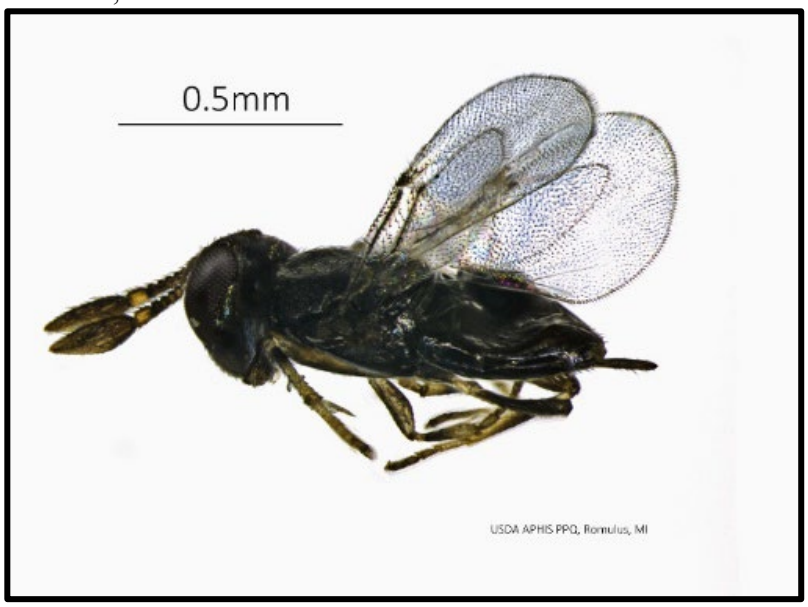


4. Smooth thorax two lateral grooves on the middle thoracic segment.

Oobius agrili checklist

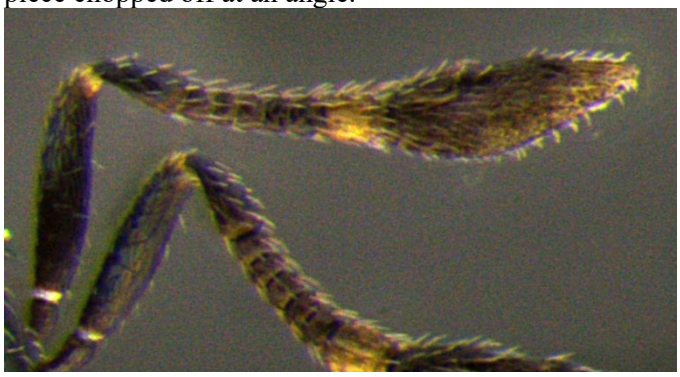
Oobius agrili can be identified by 3 characteristics: 1) Stout body, no waist, 2) Second to last antennal segment is light, all other segments are dark, 3) Clava, last segment of antennae, is tapered upwards to a blunt point when the insect is viewed in profile. Depending on condition of your specimen some characteristics may be hard to see or confirm, you can ship any suspect specimen you are unsure about for confirmation.

1. Stout, no waist



2. Second to last antennal segment light (see arrow), all other segments dark.

3. Clava tappers upward to a blunt point when insect is viewed in profile. It looks like the clava has had a piece chopped off at an angle.



Spathius galinae checklist

Spathius galinae can be identified by 5 to 6 characteristics. 1) Forewings-tinged brown with clear spots. Wings with brown veins, 2) Spherical head and spherical eye. Back of the head smooth and without wrinkles, 3) Base of the tibia of all legs is yellowish, 4) Pedicel- narrow waist connecting the thorax and abdomen, 5) Long antennae >26 segments. 1.2 x length of body, and 6) IF FEMALE: Long ovipositor. Depending on condition of your specimen some characteristics may be hard to see or confirm, you can ship any suspect specimen you are unsure about for confirmation.

1. Forewings tinged brown with clear spots.
Wings with brown veins.

2. Spherical head and spherical eye. Back of the head smooth and without wrinkles.

3. Base of the tibia of all legs is yellowish (see arrows)

4. Pedicel - narrow waist connecting the thorax and abdomen – is short.

5. Long antennae >26 segments. 1.2 x length of body.

6. IF FEMALE: Long ovipositor

Spathius agrili checklist

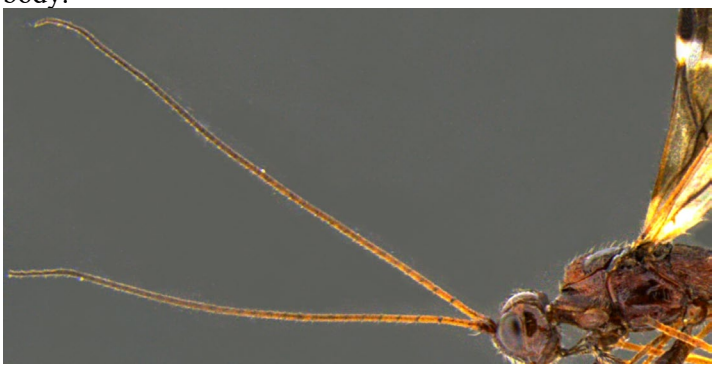
Spathius agrili can be identified by 4 to 5 characteristics. 1) Forewings-tinged brown with clear spots. Wings with brown veins, 2) Spherical head and spherical eye. Back of the head smooth and without wrinkles, 3) Pedicel- narrow waist connecting the thorax and abdomen, 4) Long antennae >26 segments. 1.2 x length of body, and 5) IF FEMALE: Long ovipositor. Depending on condition of your specimen some characteristics may be hard to see or confirm, you can ship any suspect specimen you are unsure about for confirmation.

1. Forewings tinged brown with clear banded areas. Wings with brown veins

2. Spherical head and spherical eye. Back of the head smooth and without wrinkles.

3. Pedicel - narrow waist connecting the thorax and abdomen – is short.

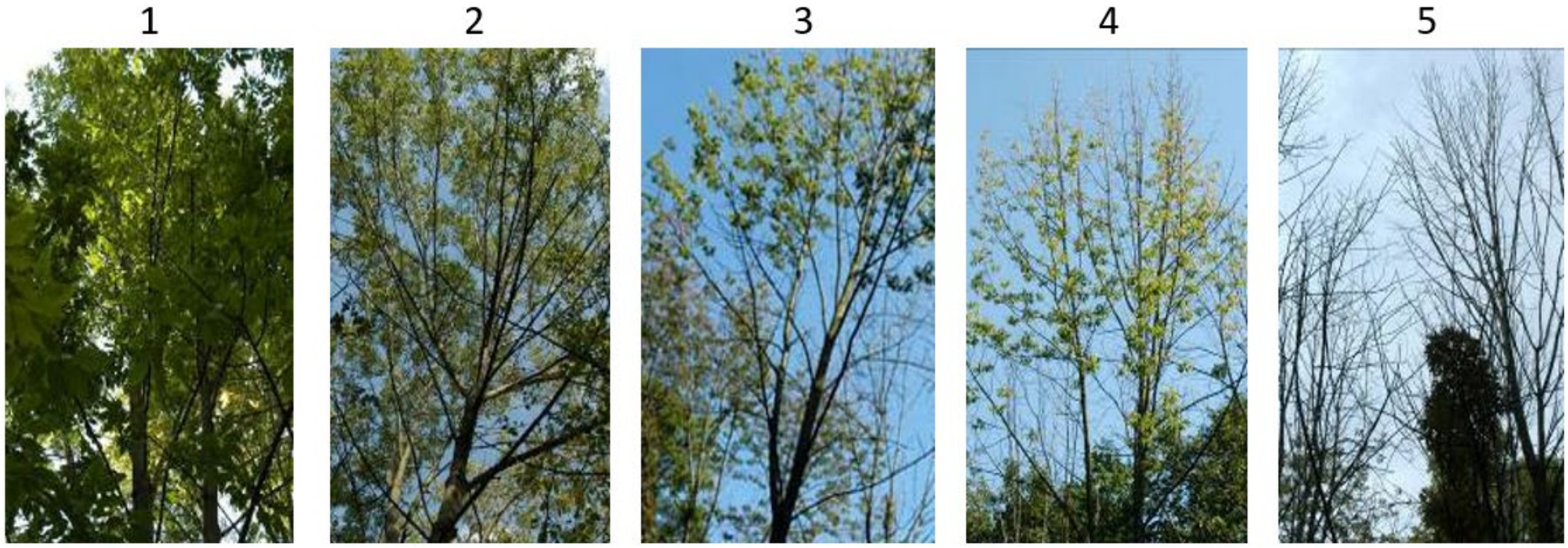
4. Long antennae >26 segments. 1.2 x length of body.



5. Abdomen very dark in color. IF FEMALE: Long ovipositor

Crown-class condition scale for ash trees infested with EAB. From Smith, A. 2006. Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River watershed in southeast Michigan. M.S. Thesis, The Ohio State University.

Canopy health is rated from 1-5 to estimate level of EAB infestation. 1 a healthy crown or canopy with no signs of decline, 2 a tree with minor signs of canopy decline, 3 moderate signs of canopy decline, 4 major signs of canopy decline and 5 a dead tree.



MapBioControl (to enter release and recovery data):

www.mapbiocontrol.org

Growing Degree Days:

http://uspest.org/US/

EAB Program Manual:

https://www.aphis.usda.gov/sites/default/files/eab-manual.pdf

APHIS Emerald Ash Borer Home Page:

https://www.aphis.usda.gov/plant-pests-diseases/eab

Emerald Ash Borer Network:

http://www.emeraldashborer.info/

Hungry Pests: The Emerald Ash Borer:

https://www.aphis.usda.gov/plant-pests-diseases/eab

Questions and Answers: Biological Control for Emerald Ash Borer

https://www.aphis.usda.gov/sites/default/files/faq_eab_biocontrol.pdf

Questions and Answers: Release and Recovery of Biological Control for Emerald Ash Borer

https://www.aphis.usda.gov/sites/default/files/qa-eab-release-and-recovery.pdf

Spathius agrili is expected to establish in areas with EAB undergoes a one-year life cycle, while *T. planipennisi* and *S. galinae* are expected to establish in areas where EAB has a two-year life cycle. We collected data on overwintering of EAB at 69 sites in 21 states to find the range of one-year and two-year life cycle of EAB in the US and modelled the proportion of EAB expected to be in a two-year life cycle. We matched this model with data on *T. planipennisi* establishment to help guide where we will release *T. planipennisi, S. galinae and S. agrili.*

- *Tetrastichus planipennisi* will be preferentially released at locations that accumulate fewer than 3,500 GDD50F between January 1 and September 30.
- If your site accumulates > 3,500 GDD 50F in the summer and you would still like to release *T. planipennisi*, confirm that you have 3-4th-instar EAB larvae in late winter or early spring before scheduling *T. planipennisi* releases.
- We rarely see establishment of *T. planipennisi* at sites where >3,975 GDD 50F accumulate in the summer, and we do not recommend releasing this species in these locations.
- We will follow the same guidelines for releasing *Spathius galinae.*
- *Spathius agrili* will only be released at sites that accumulate > 3,500 GDD 50F. It has not established in areas with fewer GDD.

Table F1: Maximum threshold for accumulated growing degree days for Jan. 1 – Sept. 30 (base 50˚F) to achieve a given predicted probability of EAB overwintering as larvae. For each predicted probability of EAB larvae we also show the percentage of sites where *Tetrastichus planipennisi* was released and establishment occurred.

Modelled percentage EAB overwintering as larvae	Growing Degree Day Threshold	Percentage of sites samples with *T. planipennisi* establishment
51-80%	2,985	92%
26-50%	3,500	78%
11-25%	3,975	50%
0-10%	-	23%

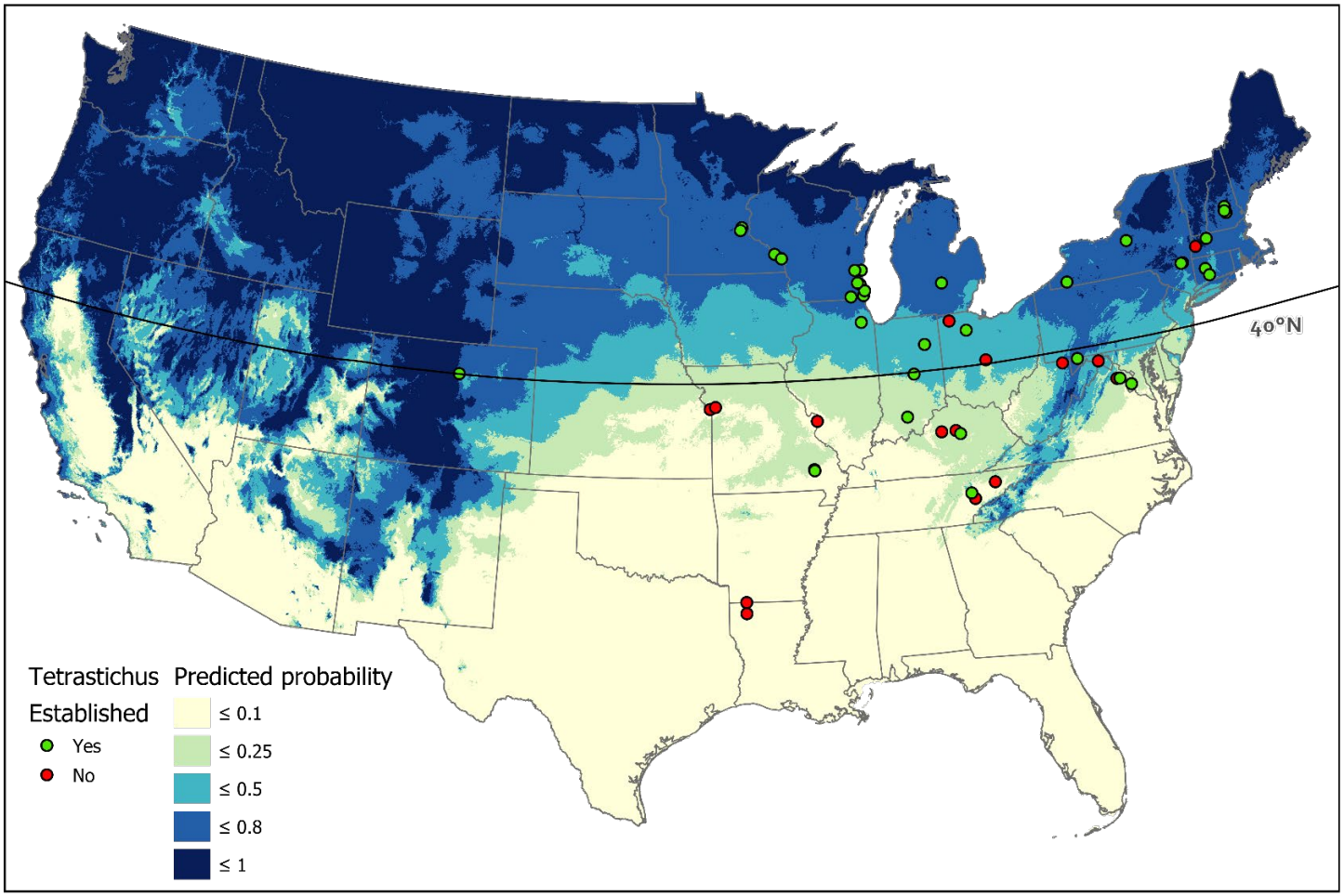


Figure F1. Predicted proportion of EAB that spend the winter as larvae instar 1-4, not as J-larvae. Locations where *Tetrastichus planipennisi* has been collected two or more years following the final release are indicated in green. Locations where samples were collected but *T. planipennisi* was not recovered are marked in red.

Appendix G – Parasitoid release datasheet

SITE ID	DATE	TIME	PARASITOID	# RELEASED	LIFE STAGE	LONGITUDE	LATITUDE	WEATHER	WIND SPEED	TEMP (F)	COMMENTS
5468	16-Aug-23	10:30 AM	Oobius agrili	200	Pupae	44.836317	-74.7611	Rain/Cloudy	Not reported	66	example entry

Stage: (Adult, Pupae, Both) Weather: (Sunny, Partly Cloudy, Foggy, Light Rain, Moderate Rain, Heavy Rain, Thunderstorms) Wind: (Light, Moderate, Strong)

Appendix H – Ash tree peeling datasheet

Location: _____ Date _____ Peeler Name _____

					Tally each gallery as you peel, enter total at end						
Site ID #	Tree #	Log # (opt-ional)	DBH	LAT/ LONG	# Live EAB	# Broods parasitized by Tets (save & ship)	# Broods parasitized by Spats (save & ship)	# Broods Parasitized by unknown (save & ship)	# Broods parasitized with solitary larvae, cocoon or pupa (do not ship)	Recovery #	Comments
					Total:	Total:	Total:	Total:			
					Total:	Total:	Total:	Total:			
					Total:	Total:	Total:	Total:			
					Total:	Total:	Total:	Total:			

Appendix I – Recovery of parasitoids from yellow pan traps datasheet

Site ID #	**State**	**Date Sampled:**	Pan Trap # *(Optional)*	**# Spat agrili**		**# Spat galinae**		**# Tet-rastichus**		**# Oobius argili**	**Recovery ID #**	Vial # *(Optional)*	EAB *(Optional)*	Native Para *(Optional)*	**Processor Name:** **Comments**
				M	**F**	**M**	**F**	**M**	**F**						
5468	**NY**	**15-Jun-23**	**5**				**1**				**4324**				**Example entry**

Please include your recovery #, site #, state and date <u>in pencil on a paper label inside each vial you keep</u>. Recovery # is generated when you enter the recovery on mapbiocontrol. If you have two vials with the same recovery # you can add a letter e.g. 43624-A and 43624-B.