

United States
Department of
Agriculture

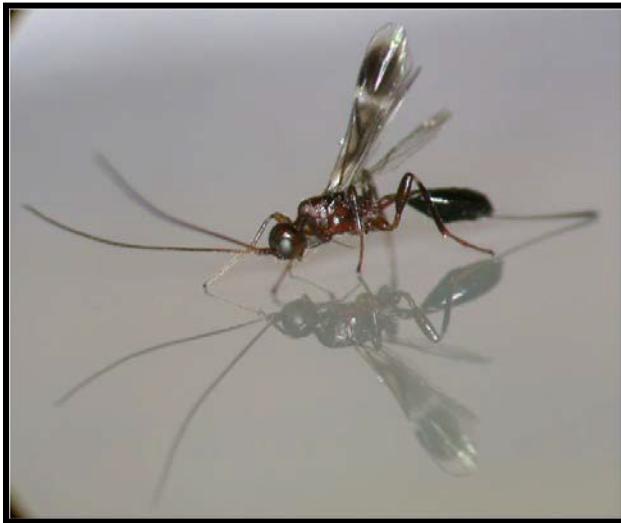
Animal and
Plant Health
Inspection Service
Plant Protection
and Quarantine/

Agricultural Research
Service

US Forest Service

Cooperating State
Departments of
Agriculture

Emerald Ash Borer Biological Control Release and Recovery Guidelines



Emerald Ash Borer, *Agrilus planipennis* (Fairmaire), Biological Control Release and Recovery Guidelines 2020

Prepared by:

Juli S. Gould, Entomologist and Theresa Murphy, Biological Technician
USDA APHIS PPQ S&T Laboratory Juli.R.Gould@usda.gov
508-563-0923

Benjamin Slager,
USDA APHIS PPQ, EAB Biocontrol Rearing Facility Benjamin.h.slager@usda.gov
810-844-2704

Leah S. Bauer, Research Entomologist Emeritus
USDA Forest Service, Northern Research Station leah.bauer@usda.gov
517-884-8059

Jian Duan, Research Entomologist
USDA ARS Beneficial Insects Introduction Research Unit Jian.Duan@usda.gov
302-731-7330 ext. 249

Toby Petrice, Entomologist
USDA Forest Service, Northern Research Station toby.petrice@usda.gov
517-884-8058

ACKNOWLEDGEMENTS

Substantial contributions to the methods for rearing parasitoids and conducting field sampling were provided by: Ben Slager, Tracy Ayer, Houping Liu, Debbie Miller, Phil Taylor, Kristopher Abell, Jason Hansen, and Michael Ulyshen.

Thank you to the following for providing critical input in the development of this document: Joe Beckwith, James Buck, Russ Bulluck, Sharon Lucik, Michael Winks.

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

Cite this report as follows: USDA–APHIS/ARS/FS. 2020. Emerald Ash Borer Biological Control Release and Recovery Guidelines. USDA–APHIS–ARS–FS, Riverdale, Maryland

Contents

ACKNOWLEDGEMENTS	I
INTRODUCTION BRIEF HISTORY OF EAB IN NORTH AMERICA	1
LIFE-CYCLE OF EAB.....	1
DAMAGE AND SIGNS OF INFESTATION	3
ECONOMIC CONSEQUENCES OF EAB INFESTATIONS	3
HOST RANGE OF EAB	4
BIOLOGICAL CONTROL OF EMERALD ASH BORER	5
BIOLOGY OF EAB BIOCONTROL AGENTS.....	5
REARING EAB PARASITIDS.....	7
PROJECT STATUS	8
PREPARATION FOR PARASITOID RELEASE	9
FIELD RELEASE	10
OUTLINE OF PROCEDURES FOR EAB BIOCONTROL RELEASES.....	10
RELEASE SITE SELECTION	11
PRE-RELEASE SITE ASSESSMENT	14
PRE-RELEASE SITE PREPARATION	16
RELEASE OF PARASITIDS	16
RELEASE CONSIDERATIONS: WHICH SPECIES, WHERE, WHEN	16
<i>Tetrastichus planipennisi</i>	17
<i>Spathius galinae</i>	18
<i>Spathius agrili</i>	19
<i>Oobius agrili</i>	19
REQUESTING PARASITIDS	19
RECEIPT OF PARASITIDS	20
CARE OF PARASITIDS IF RELEASE IS DELAYED	21
TRANSPORTING PARASITIDS TO FIELD SITES.....	22
RELEASE OF PARASITIDS	22
ENTER RELEASE DATA	23
EVALUATING PARASITOID ESTABLISHMENT	24
TREE FELLING AND DEBARKING.....	26
DEBARKING	27
<i>Recovering the Egg Parasitoid Oobius from Outer Bark Samples:</i>	27
<i>Peeling Logs to Recover Larval Parasitoids:</i>	30
YELLOW PAN TRAPS.....	31
<i>Processing Yellow Pan Trap Samples</i>	37
ENTER RECOVERY DATA	42
PHOTO GUIDE TO IDENTIFYING EAB PARASITIDS IN YELLOW PAN TRAPS	45
<i>TETRASTICHUS PLANIPENNISI</i> (EULOPHIDAE)	45
<i>OBIUS AGRILI</i> (ENCYRTIDAE).....	49
<i>SPATHIUS</i> SPECIES (BRACONIDAE).....	51
<i>ATANYCOLUS</i> SP.	54

Appendix A- EAB Life Stages and Damage
Appendix B- Parasitoid Life Stages
Appendix C- Crown Condition of Ash Trees
Appendix D- Helpful Links
Appendix E- Releasing Parasitoids for Optimal Establishment

INTRODUCTION 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 packing material originating in Asia.

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 2020, 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, 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. At present, the most sustainable and long-term approach to reducing EAB populations and conserving ash in forested areas of North America is biological control.

LIFE-CYCLE OF EAB

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

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 phloem and outer layer of new sapwood where they feed until the weather gets too cold in the fall. There are four stages of larval development (instars) (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, 4th-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. Mature larvae complete development (i.e. become an adult beetle) the next spring, whereas younger larvae may require another summer of feeding, becoming adult beetles the following spring.

DAMAGE AND SIGNS OF INFESTATION

EAB larvae damage ash trees by feeding on the phloem. 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). 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). 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).

ECONOMIC CONSEQUENCES OF EAB INFESTATIONS

The cost of managing EAB is already high. On average, federal and state resource managers spend at least \$29.5 million per year to manage EAB populations. The compensatory value of the 8 billion ash trees in U.S. timberland potentially infested with EAB is \$282 billion. States in the eastern U.S. produce nearly 114 million board feet of ash saw timber annually, with a value of \$25.1 billion. White,

black, and green ash make up >7 percent of the hardwood stand mix and 5.5 percent of the total stand mix (including conifers) in the northeastern United States and eastern Canada. The 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. The continued spread of EAB threatens our ash resources and will permanently alter forest ecosystems in North America. The 16 native species of ash, some with limited distributions in North America, are now threatened by EAB.

In addition to its value to the timber industry and the forest ecosystem, ash was one of the most popular landscape trees because of its tolerance of a range of environmental conditions and resistance to pests. Ash was the most commonly planted tree species used to replace elm trees decimated throughout North America by Dutch elm disease and for new residential and commercial developments. The estimated cost of treating, removing, and replacing 37.9 million ash trees in urban and residential settings in 25 states for one decade (2009-2019) was estimated at \$25 billion.

Nationwide, the nursery industry produced an estimated 2 million ash trees each year. With median approximate values ranging from \$50 to \$70 per tree, the annual ash nursery stock was worth between \$100 and \$140 million.

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*), and blue ash (*F. quadrangulata*). 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. EAB may attack white fringetree and commercial olive, but only when adjacent to heavily infested ash,

furthermore, evidence suggests they recover in the absence of EAB.

BIOLOGICAL CONTROL OF EMERALD ASH BORER

Biological control (or biocontrol) is the practice of importing and releasing natural enemies from a pest's native range to control the target pest populations in the area of introduction. Biocontrol has been used for over 100 years in the U.S. and has successfully controlled invasive plant and insect pests such as gypsy 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 due to the combined effects of EAB-resistance in Asian ash species, scarcity and patchiness of forests, and the EAB natural enemy complex. Exploration for EAB natural enemies in China, Russia, and Korea has yielded several hymenopteran parasitoids, and four species have been approved for release as biological control agents of EAB in the U.S and others 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, thus 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 which modelling predicts will be in areas that accumulate more than 3,500 GDD 50F (Appendix E). Female *S. agrili* 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. 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 planipennis is another larval parasitoid of EAB collected from China. The life cycle of *Tetrastichus* is similar to that of *Spathius agrili*, however, the female parasitoid lays eggs inside EAB larvae where the parasitoid larvae grow, eventually killing their 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 E).

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 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. *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 PARASITIDS

The USDA APHIS PPQ Biological Control Production Facility in Brighton, MI produces EAB parasitoids for field release. This facility is also called the EAB Biocontrol Rearing Facility and is also referred to as the EAB Biocontrol Program. 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 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. There are also challenges storing the *Spathius* species in chill for stockpiling prior to release.

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 as adults in plastic cups with solid caps. *Oobius* pupae are released by attaching the Oobinators to ash trees, with the screen-side down, and removing the plastic cap. 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.



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. These bolts will be shipped with a small hole drilled through the top to provide a point of attachment to a release tree. Twine or zip ties are common materials used to attach release bolts.

Spathius galinae will be shipped exclusively as adults in

cups due to the difficulty in getting *S. galinae* to emerge from bolts that have been kept in storage for several months. *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.



PROJECT STATUS

Since EAB parasitoid releases began in 2007, researchers have found that *Tetrastichus planipennisi* and *Oobius agrili* have established in many states and provinces, are dispersing from the release sites, and are responding to changes in EAB density by increasing percentage parasitism. *Spathius agrili* is found periodically at some southerly release sites, but we are still in the early stages of release in the south. Release of *Spathius galinae* began in 2015, and its establishment and spread appear successful at long-term study sites in Connecticut, Massachusetts, Michigan, and New York. Another species of *Oobius*, reared from EAB eggs collected in Russia, is being evaluated as an EAB biocontrol agent for some regions of the U.S. and Canada. Over the last 10 years, the EAB Biocontrol Rearing Facility in Brighton, Michigan has produced and released more than 6 million parasitoids. Releases have occurred in 29 states (Arkansas, Colorado, Connecticut, District of Columbia, Delaware, Iowa, Illinois, Indiana,

Kansas, Kentucky, Louisiana, Massachusetts, Maryland, Maine, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Dakota, Tennessee, Virginia, Wisconsin and West Virginia) and 3 Canadian Provinces (New Brunswick, Ontario and Quebec).

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 PPQ 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 is 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. 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 are encouraged to use the MapBio app (available for iPads and iPhones) or they can also use a hand-held GPS device and/or hard copy datasheets to collect data in the field. The data from the MapBio app can be synchronized (uploaded to mapbiocontrol.org). Data written on hardcopy datasheets and hand-held GPS units must be entered online manually.



FIELD RELEASE

OUTLINE OF PROCEDURES FOR EAB BIOCONTROL RELEASES

- **It is no longer necessary** to obtain release permits. The EAB Biocontrol Rearing Facility has permits to release all four parasitoid species in all of the states currently infested with EAB.
- **Select a release site** in an area with good access, high density of ash trees of various sizes, and infested with EAB.
- **Obtain Local Land-Use Permits**
- **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** using an iPad/iPhone (you will need to download the MapBio app from the Apple Store) or manually enter the information into 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.
- **Synchronize your iPhone.** If you are using an iPhone/iPad, the unit should be synchronized with MapBioControl.org by selecting send/receive data every time data are collected to prevent the loss of data or data can be entered manually.
- **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 your iPhone/iPad with MapBio app installed 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 the MapBio app on your iPhone/iPad or online at mapbiocontrol.org within 48 hours of release.
- **Assess Parasitoid Establishment:** Determine if the parasitoids are established in each general release area (not necessarily at each site) **at least one year following the Year 2 release** (Year 3 or more). This should be done during the fall, winter, or early spring (for bark sampling or log debarking) or late spring, summer, and early fall (for yellow pan traps). Several methods are available for parasitoid recovery, with the choice of method depending on the specific circumstances of each release site.

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 E). The site information described below should be collected and entered into mapbiocontrol.org. This information will help researchers and the EAB Biocontrol Rearing Facility Manager determine which sites are most suitable for release and establishment of the parasitoids.

The guidelines below outline the best practices for EAB parasitoid 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. For example, the site may have low acreage, but its connectivity to other plots is high. If determining the suitability for a particular site is not straightforward, please email the rearing facility (EAB.Biocontrol.Program@usda.gov) to discuss that you have found what you believe to be the most suitable release site for your county, even though it doesn't meet all of the requirements below.

General Site Characteristics

Locate parasitoid-release sites in naturally forested areas, woodlots, wooded wetlands, and riparian zones. 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. Avoid sites with excessive human activity, as well as sites along roads, trails, or railroad tracks, and in picnic areas, golf courses, and open park lands. Ash trees in such public areas may be treated with insecticides or removed.

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 $\leq 25\%$, 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

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 can become 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 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, we recommend using an indirect EAB-density estimate based on the signs and symptoms of EAB infestation in 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 C). 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.

Access and local use permits

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 from land owners for use of the site to both release parasitoids and conduct recovery activities over a period of five years.

Keep in mind that it may take months to obtain permission or land-use permits from land owners or park managers.

PRE-RELEASE SITE ASSESSMENT

Note: The MapBio app will work on an iPad but you need to search “iPhone only” apps to find it. It will not appear under iPad apps. Also some iPads have cellular service and GPS capabilities but not all do. You will need an iPad with a GPS to properly use the MapBio app.

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. General Site Details and Physical Details data can be collected with an iPhone or iPad (MapBio app) and can also be entered online at www.mapbiocontrol.org. The mapbiocontrol app, MapBio, can be downloaded onto iPhones/iPads to simplify data collection and entry. When collecting data using an iPhone/iPad, 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 collected on the iPhone/iPad are synchronized by clicking send/receive data, 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 Staff and state cooperators prioritize and select 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)

PRE-RELEASE SITE PREPARATION

General Plot Design and tree selection

After a site is chosen for parasitoid release, locate the center of the release plot generally in the middle of the trees infested with EAB. Positive signs of EAB include woodpecker feeding, bark splits, epicormic shoots, poor crown condition, and/or D-shaped exit holes. This will be the parasitoid-release epicenter.

Release trees should be large enough to support hanging ash bolts containing parasitoids and should be spread throughout the release plot.

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 areas and they are not all suitable for releases in all states. Thus, we have made new recommendations based on current research results (Appendix E).

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 EAB 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 not always accessible without felling trees. Spreading releases out over multiple weeks should help ensure that the proper stages of EAB eggs and larvae are present for parasitism to occur. Growing degree day accumulations and forecasts can be found at <http://uspest.org/US/> and <http://uspest.org/cgi-bin/ddmodel.us> to assist with timing EAB parasitoid releases.

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. While in past years we suggested that the 40th parallel generally predicts this demarcation, we now have a model that delineates the areas expected to have over 25% EAB overwintering as larvae shown in Appendix E.
- ❖ If your site accumulates > 3,500 GDD50F 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. 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 ash trees and branches less than 6 inches in diameter at your site that contain EAB larvae before releasing *T. planipennisi*. Also, saplings and branches need to be over 1 inch in diameter to support EAB larvae.
- ❖ 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 your site does 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 in the spring and *T. planipennisi* will not be able to establish.
- ❖ 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 United States 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 your site does 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 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 6 weeks (1,200 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). If you have an emergency, please contact the Biological

Control Release Coordinator – Scott Whitehead (810-844-2708) or the Facility Manager – Ben Slager (810-844-2704). You should enter data on the site characteristics and parasitoid releases into MapBioControl.org. Note: the Request Tab for parasitoid requests is currently under development and should not be used at this time.

RECEIPT OF PARASITIDS

Parasitoids are shipped by overnight delivery in a cardboard box, and should arrive by 10:30 AM 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. Ash bolts will have a hole drilled through the log as a point of attachment for mounting on release trees. Twine, zip ties, and nails are commonly used attachment materials but are not included in parasitoid shipments. Honey will be smeared on the screening as a source of food for the adult parasitoids in cups. 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 tissue paper. *Oobius* may also 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 prior to 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 nails.

The parasitoids should be released soon after receipt. All parasitoids should be released on the day they are received. If you are unable to release parasitoids as scheduled because of personnel shortages or expected adverse weather conditions, contact the Biological Control Release Coordinator to arrange for a different shipping date. 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 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 a number of 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 a lid and with moistened paper toweling. Before placing the cups in the plastic tub, 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 screening 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, do not re-bag for local transport, but keep the cups or infested logs in the original boxes. Care should be taken to keep the box out of direct sunlight or other potentially hot (e.g., a sealed vehicle) environments. 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 at all times*** 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. Parasitoids are extremely small and 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. 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 parasitoids that refuse to leave the cup but be very careful not to hurt the parasitoid in the process. 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 come with a pre-drilled hole through which you can insert a long wide head nail (4-5”) to pound into the tree trunk or use a shorter wide head nail and a zip-tie or wire to hang the log from the trunk. You can also use zip tie or wire to hang the bolts to branches of smaller trees. **You will need a hammer, nails, and/or zip ties or wire to hang the bolts in the field.** Other options for hanging the bolts include tapping a nail into the top of the bolt and hanging the bolt from a short piece of wire, wrapped around a horizontal branch of the tree – this is particularly effective on large trees where trunk diameter makes it difficult to hang the bolt on the side of the trunk. Do not hang more than one bolt per tree if possible; the parasitoids will establish better if they are spread out. The bolts should remain in the field for at least 6 weeks to assure that all the parasitoids have emerged as adults. **Remove the nails from the trees when you recover the infested 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*. 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 6 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 this data the day you conduct your releases, as you will quickly forget what the weather was like if it is not recorded. **We ask that you enter release data using the MapBio app on your iPhone/iPad or online**

at mapbiocontrol.org within **48 hours of release**. Every time you release parasitoids, enter the following information into your MapBio app and synchronize/upload it, or these data can be recorded on datasheets in the field 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 Female *Oobius* Released** (when releasing as pupae this will be an estimate)
- **Stage *Oobius* released** (Adult, pupae, both)
- **Number Female *Spathius agrili* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Spathius agrili* released** (Adult, pupae both)
- **Number Female *Spathius galinae* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Spathius galinae* released** (Adult, pupae both)
- **Number Female *Tetrastichus* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Tetrastichus* Released** (Adult, pupae, both)
- **Note**

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.

To confirm establishment, sampling should be done at least one year after the final release at a given site. 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 planipennis 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 during spring, summer, and fall, although they are most abundant in late summer in northern areas.

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 larva 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). *Spathius agrili* and several native species of

Spathius require a period of chill to break diapause and emerge as adults, which are preferable for identification. For adult parasitoids to emerge from cocoons, we recommend felling trees for debarking after December (see the section below “**Peeling Logs to Recover Larval Parasitoids**”). If you would like to determine whether a recovered *Spathius* is one of the released species, you can remove a parasitoid larva from a cocoon, place it in 95% ethanol, and ship it to Juli Gould, 1398 West Truck Road, Buzzards Bay, MA 02542 for molecular identification. Like *Tetrastichus*, adults of *S. agrili* and *S. galinae* can also be recovered in yellow pan traps.

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 dark brown or black 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. See the DEBARKING section below, as well as an online PowerPoint presentation with instructions and photographs at:

https://www.mda.state.mn.us/sites/default/files/inline-files/barksifting2016_0.pdf 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 (EAB.Biocontrol.Program@usda.gov). If you have questions about parasitoid identification, recovery methods, where to purchase supplies and/or questions about how to construct, yellow pan traps, or emergence tubes, please email or call one of the authors.

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 near the release epicenter (vicinity of the original release trees) 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 diameter breast height (DBH). Give each tree a unique ID number, and record its DBH, location (GPS coordinates), and the date the tree was felled.

DEBARKING

Before debarking the logs to recover larval parasitoids, use a draw knife to gently remove a thin layer of outer bark to collect EAB eggs.

Recovering the Egg Parasitoid Oobius from Outer Bark Samples:

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 are good evidence that the tree is infested with EAB. 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, tightly 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 labelled large paper lunch bag, and return it to the laboratory.

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



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.

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.

Sifting *O. agrili* from bark samples: Dry the sample at least one month at room temperature (if 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 or magnifying glasses, sort and collect all EAB eggs and small adult parasitoids into a small Petri dish with friction-fitted lid (Fisher Scientific 50 X 9-mm dishes – catalog number 08-757-105) labelled with state, site name, and date of collection using a fine-tip permanent marker. This method, including many photos of parasitized EAB eggs and look-alikes, is described at the following Minnesota Department of Agriculture webpage: <https://www.mda.state.mn.us/sites/default/files/inline-files/barksifting2016.pdf>. For confirmation or identification, ship the EAB eggs and parasitoids collected from bark sifting or reared from bark or logs to Juli Gould, USDA-APHIS, 1398 West Truck Road, Buzzards Bay, MA 02542.



Bark samples to recover eggs can also be taken from ash trees felled for other recovery work. We suggest taking one bark sample per tree from flaky bark from a log cut from the lower two meters of each tree. You are more likely to find EAB eggs by sampling the 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). From this part of the lower trunk mark, mark off a vertical area of bark 10 x 100 cm, debark this area of bark with a drawknife onto a piece of heavy plastic, and pour each bark sample into a large paper lunch bag, label, and rear or sift the bark samples as described above. Sample at least 10 trees per site.

Peeling Logs to Recover Larval Parasitoids:

Both species of larval parasitoid can be found in EAB galleries under the bark (for more information on the best time of year to sample trees, see the section above “*Spathius agrili* and *Spathius galinae*”). Logs are easiest to peel if debarked soon after felling, but if you need to store the ash



logs in a cold chamber, consider sealing the ends (with Anchorseal[®] for example) to reduce moisture loss or keeping the logs in a barrel of water. If the bark is thick, remove the outer bark with a draw knife first. It is easiest to peel using a large 9-15” drawknife but if you find an EAB gallery you can more carefully remove the phloem in that area with a sharp chisel or small 5” drawknife. 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. Inspect all EAB galleries for signs of parasitized larvae, including empty galleries from which *Tetrastichus* has already emerged (see Appendix B for photos of parasitized EAB). *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 them. Many small eggs and parasitoid larvae can be seen swimming in the EAB haemolymph (See appendix B for helpful dissection photos). If EAB parasitism is suspected, 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). Using a fine-tipped permanent marker, label each Petri dish with the state, site, tree number, and date. Mail the specimens within one week to Juli Gould, 1398 West Truck Road, Buzzards Bay, MA 02542 for identification. For each tree, record the number of live EAB larvae, the number of solitary larvae or cocoons (probably *Atanycolus* – just count, do not ship), the number of broods of gregarious larvae (ship these for identification) and the number of broods of gregarious cocoons and pupae (ship these for identification). You do not have to count the number of parasitoids in each gallery. Enter these data into MapBioControl. The identifiers will enter the final identification data into MapBioControl.

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 sulcata*, *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.

Over the years we have found pan traps work really well in some situations and not so well in others. We still do not fully understand where they are most useful, but our general thoughts are as follows. A

lot of the success of the traps 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 including more replicates. We have been investigating where the yellow pan traps are most successful and our thinking now 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). We have 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 also *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 in importance to finding fresh woodpecker damage and live phloem. Due to the dynamics of our forested areas it's 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 do the best you can to find live but infested trees on which to hang the traps.

What will I need to make and deploy one YPT?

1. Two 12-oz yellow plastic bowls (color: yellow sunshine; can be purchased from Partycity.com)
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) propylene glycol (non-toxic antifreeze) diluted with water.

You can search for **Food** or **USP grade** propylene glycol to find a supplier (also for sale at Amazon.com or Tractor Supply).

11. Rechargeable portable electric screw-driver with bit and extra battery pack
12. Unscented clear dish detergent

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

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.

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 $\sim\frac{3}{4}$ -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 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 wood-pecker 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.

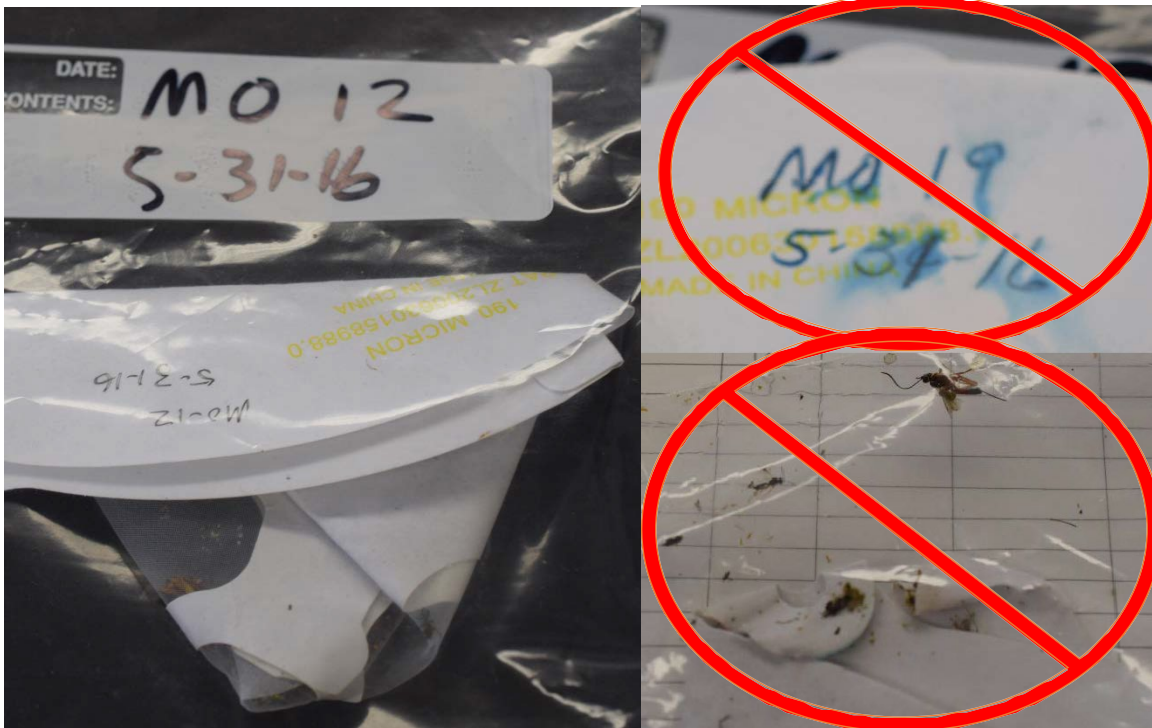
Label each YPT holding-bowl with a unique ID number using a weather-proof pen (e.g. Sharpie) or grease pencil if bowl is wet. On a data sheet, record the YPT-ID number, date, and initial of person collecting. Record the GPS coordinates – this will help you find the YPT later to recover the sample,

and it will let researchers know where the parasitoids were recovered.

When to deploy YPTs in the field? Deploy YPTs at EAB biocontrol release sites at least one year after the final parasitoid releases. Adult parasitoids fly during the spring, summer, and early fall, and the four introduced parasitoids have multiple generations per year, thus their populations are highest in mid-summer and early fall. However, the timing of possible captures in YPTs will depend on the climate in your area and the adult flight period of each species. If you have released *Tetrastichus planipennisi* and/or *Spathius galinae* and resources permit intensive sampling, we recommend deploying the YPT when 300 Growing Degree Days have been accumulated, then throughout the spring, summer, and early fall. This will require visiting sites weekly, collecting the samples, and replacing the liquid in the bowl for the next week of sampling. If resources do not permit such intensive sampling, then we recommend sampling once per month from 300 GDD50F through the end of September. In the south, where larval parasitoids are not expected to emerge during the spring, deploy the YPTs around 600 GDD50F for *Oobius* or when 3rd-instar larvae become available in the mid-late summer.

How long do I leave YPTs in the field? The YPT samples should be collected three to seven days after 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 can 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 from heat, restart weekly collections. If using this method, bowls must be filled to the overflow hole to prevent it drying out. In addition, if you get a heavy rain event, empty traps soon after because it will both displace the solution from the bowl and dilute the remaining solution.

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 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 within one or two days. If processing is delayed, store the samples in the freezer.



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.

What do I do with these samples? Because so many states are now doing recovery sampling, USDA 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 there are several native parasitoids in the genera *Spathius*, *Tetrastichus*, and *Oobius* that are easily mistaken for the EAB parasitoids. Positive identification of these parasitoids requires specialized taxonomic skills. To assist you in sorting the insects in the YPT samples, please contact the EAB biocontrol 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

Prior to processing or sorting the YPT samples, please contact the EAB biocontrol 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 to Juli Gould, 1398 West Truck Road, Buzzards Bay, MA 02542 for confirmation.

What do you need to sort through samples?

1. Dissecting microscope
2. Two pairs of fine forceps
3. A fine paint brush 0, 00, or 000 paint brush
4. 95% Ethanol (for and preserving specimens)

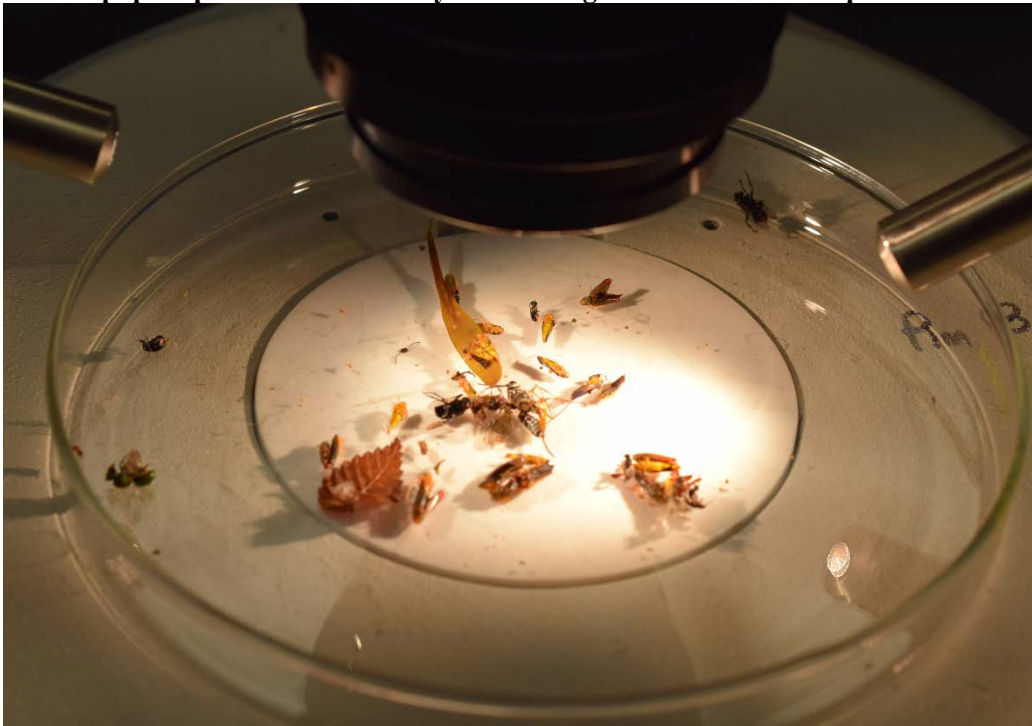
5. 70% Ethanol (for checking the samples)
6. Small screw top vials (for specimens that need confirmation ID)
7. A small dropper to help add or remove specimens from the vials (optional)
8. A glass or plastic Petri dish to hold insect samples during the sorting process

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 a 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.

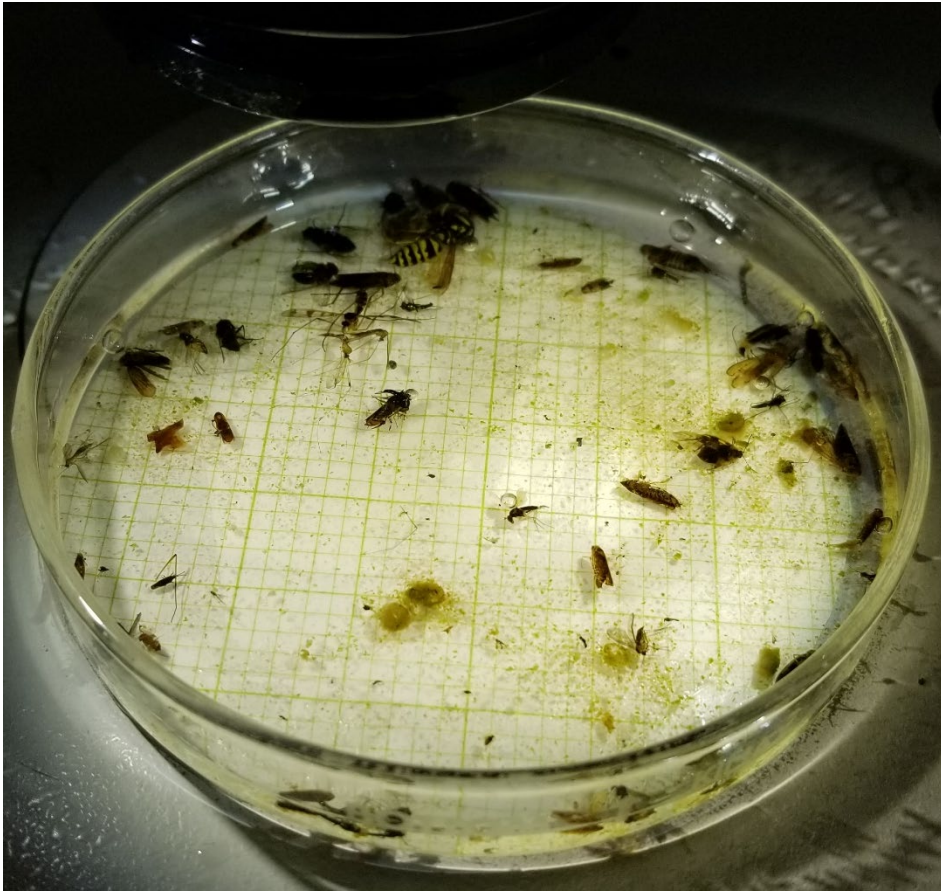
To start processing, open the zippered bag and record the location, date of the sample (including year), and pan trap number on your datasheet. Carefully unfold the filter paper, pull the filter apart at the seam, and dump the contents into your Petri dish. You may need to check the bag or outside of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



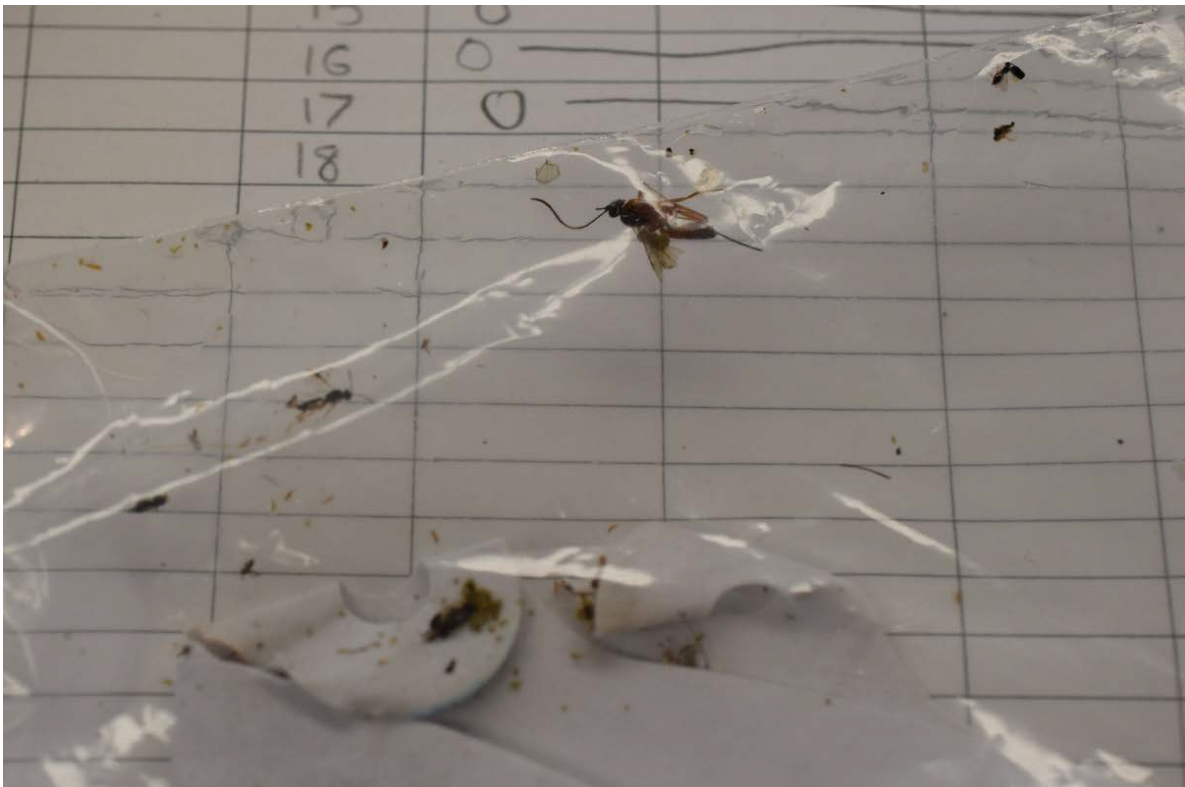
A filter paper spread out and ready for viewing under the microscope.



A sample that has been poured onto a glass Petri dish ready for viewing under the microscope.

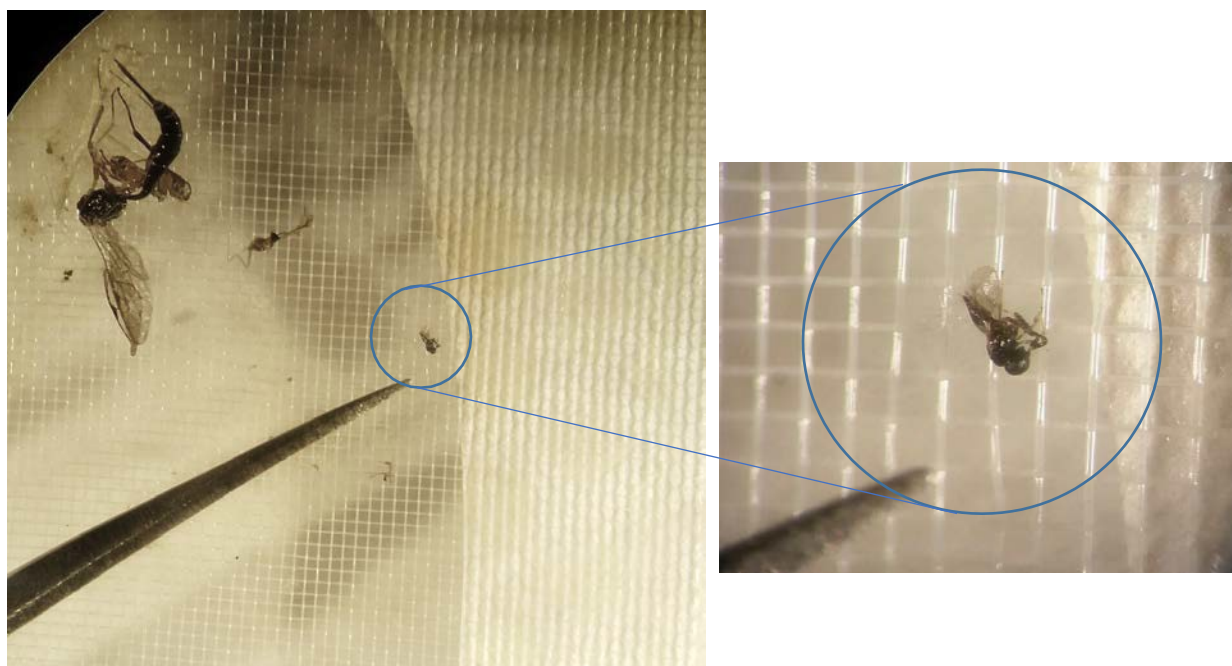


A sample where 70% alcohol has been added to help separate the insects and where grid paper has been added to help provide reference points for sorting through the sample.



Insects that have slipped out into the plastic bag. In this case view the bag under the microscope to check these insects as well.

Systematically sort through the insects caught on the filter under the microscope at the lowest magnification, looking for anything that looks similar to 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 the section below **Photo Guide to Identifying Parasitoids in Yellow Pan Traps**, as well as your reference specimens from the rearing facility. If you think it may be an EAB parasitoid, place it in a vial with 95% ethanol, include a paper label with site and date information written in pencil, and once you have accumulated a number of specimens for identification, pour most of the ethanol off each vial, and ship them overnight to Juli Gould, USDA-APHIS-PPQ, 1398 West Truck Road, Buzzards Bay, MA 02542.



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 dirt or leaves in your sample, so be sure to flip over insects and leaves and spread out dirt 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 it 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, toss away your insect and filter paper and move onto the next sample.

Place suspect parasitoids into glass or plastic screw-top vials with 95% alcohol. This alcohol must be poured off before shipping due to shipping restrictions, but alcohol will be added to the vials by the receiver. Send suspect insects to Juli Gould, USDA-APHIS-PPQ, 1398 West Truck Road, Buzzards Bay, MA 02542.

ENTER RECOVERY DATA

It is critical that the EAB Biological Control Program have data on where EAB parasitoids are 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. Data on samples that were collected but in which no parasitoids were recovered are also critical. When you enter the mapbiocontrol.org web site, 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:

- **Trap ID:** The Trap ID number in mapbiocontrol is a holdover from a previous protocol.

We do not expect you to enter each trap separately, rather enter each site and collection date once and enter the number of traps (usually 15). However, the Trap ID is a required field. This data is critical because the scientists who identify collected parasitoids need to match the

identified insects to the sites and locations where the parasitoids were recovered. We recommend that you enter a code for the release site as a place holder. If you are interested in knowing which traps collect parasitoids, make sure that the label in the vial includes the trap number.

- **Latitude** (dd.ddddd)
- **Longitude** (dd.dddd)
- **Site ID** Once you type in the Latitude and Longitude of your sample, the database will select some nearby sites from which to choose. Select the appropriate site. If you happened to find parasitoids not connected with any particular release or control site, simply select NO Site.
- **State**
- **Date Sample Collected**
- **Sample Method** (Yellow Pan Trap, Tree Debarking, Logs in Tubes, Bark in Tubes, Sentinel Eggs, Sentinel Larvae, Egg Collection, Other)
- **Number of Samples**
- **Possible EAB Parasitoids Recovered?** Yes or No

If you did recover some possible EAB parasitoids, record the date they were shipped for identification, and the person they were shipped to. The data on the number of released parasitoids recovered will be entered into the database by the identifiers.

Forest Type

On mapbiocontrol.org in the Release section, there is a tab for Forest Type. Collecting Forest Type 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 establishment of EAB parasitoids.

Ash Health Assessment

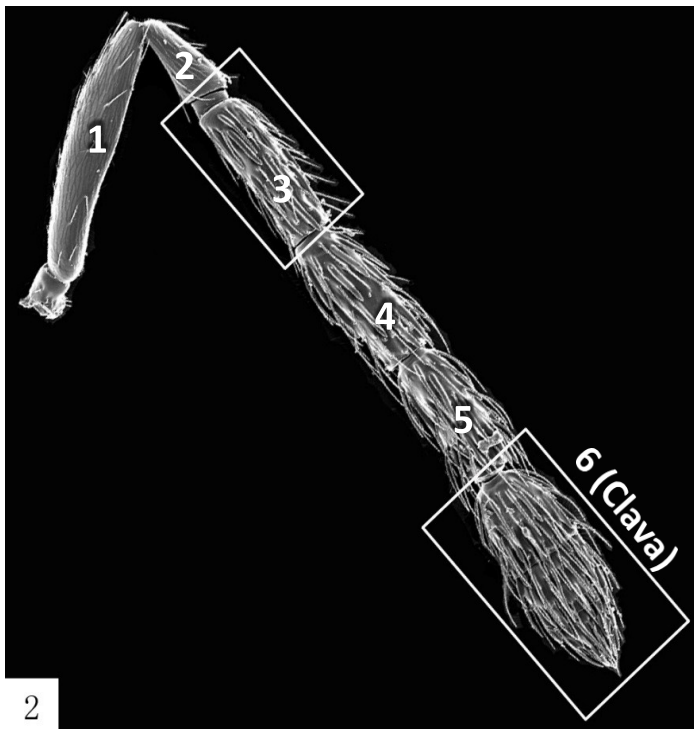
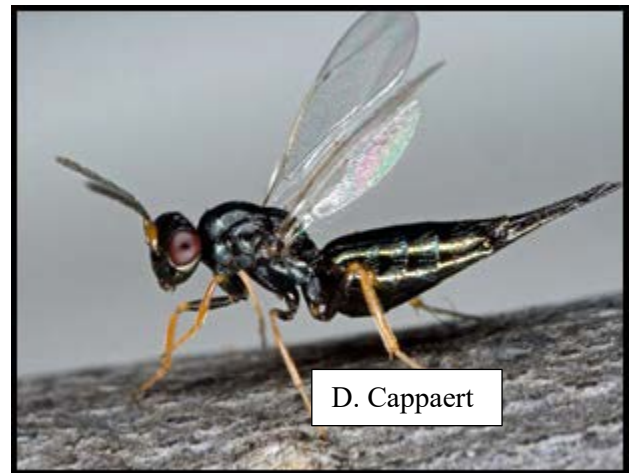
On mapbiocontrol.org in the Release section, there is a tab for Ash Health Assessment. Collecting Ash

Health Assessment data is not required, but if you have the time and resources it will greatly assist researchers in determining the trajectory of ash mortality and how it correlates with the establishment of EAB parasitoids.

Photo Guide to Identifying EAB Parasitoids in Yellow Pan Traps

Tetrastichus planipennis (Eulophidae)

A **eulophid** wasp with red to dark red eyes and a tapered shape (females). Do not go by size alone as they can get very small if they are from a particularly large brood.



Tetrastichus Female

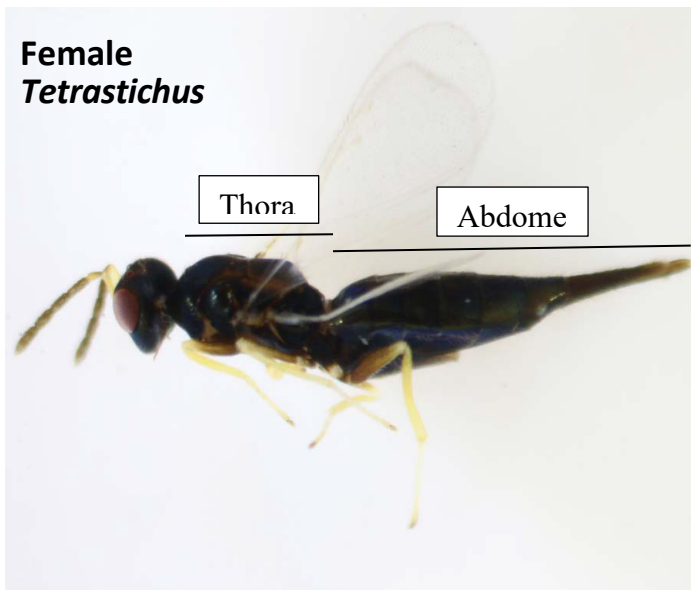


The female's antennae have 6 segments: scape (1), pedicel (2), three funicles (3-5) and 1 clava (6). The clava segment is more clubbed and robust (citation Yang et al. 2006).

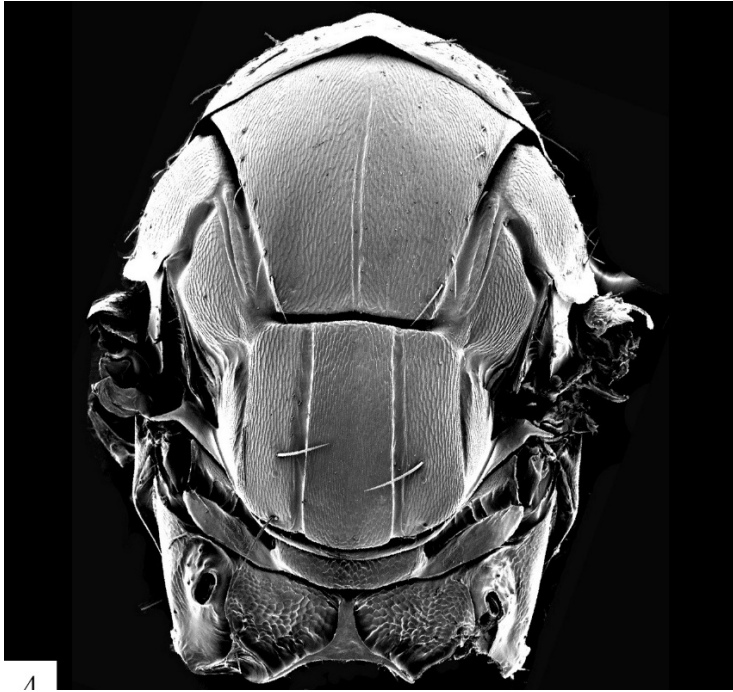


12

The male's antennae have 7 segments: scape (1), pedicel (2), 4 funicles (3-6) and 1 clava (7). The clava/last segment is thin and long. The antennae also are quite hairy, with the hairs having the look of eyelashes (much more evident in dead specimens than in these photos) (citation Yang et al. 2006).



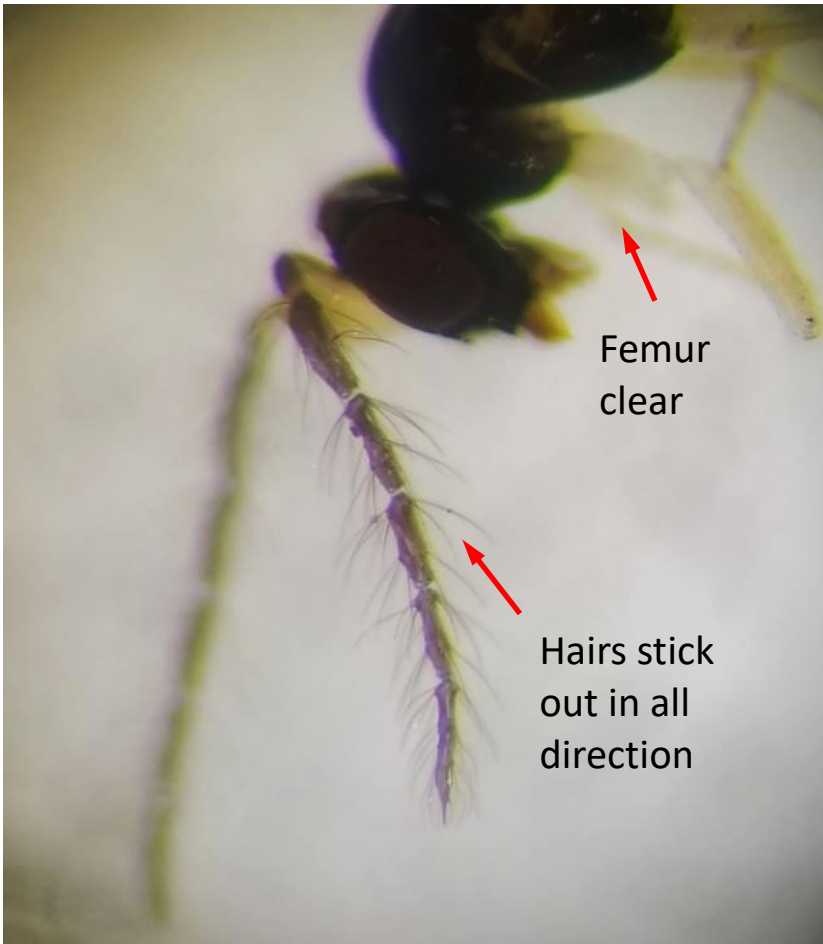
The female's abdomen is unique in that the final segment is very long. It is twice as long as the rest of the body (head plus thorax) and the abdomen is four times as long as it is wide.



Both sexes have two pairs of longitudinal grooves on the middle thoracic segment, a smooth thorax, and a dark femur.

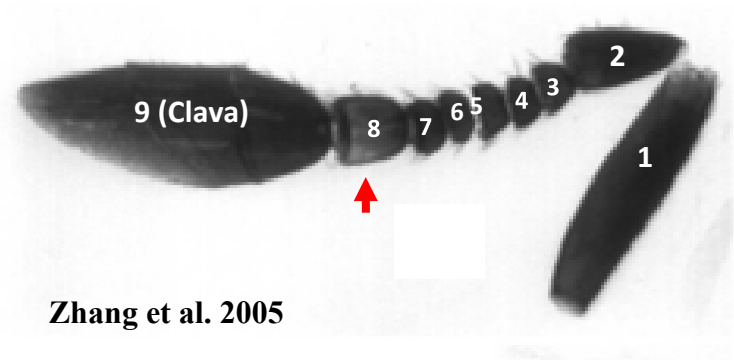
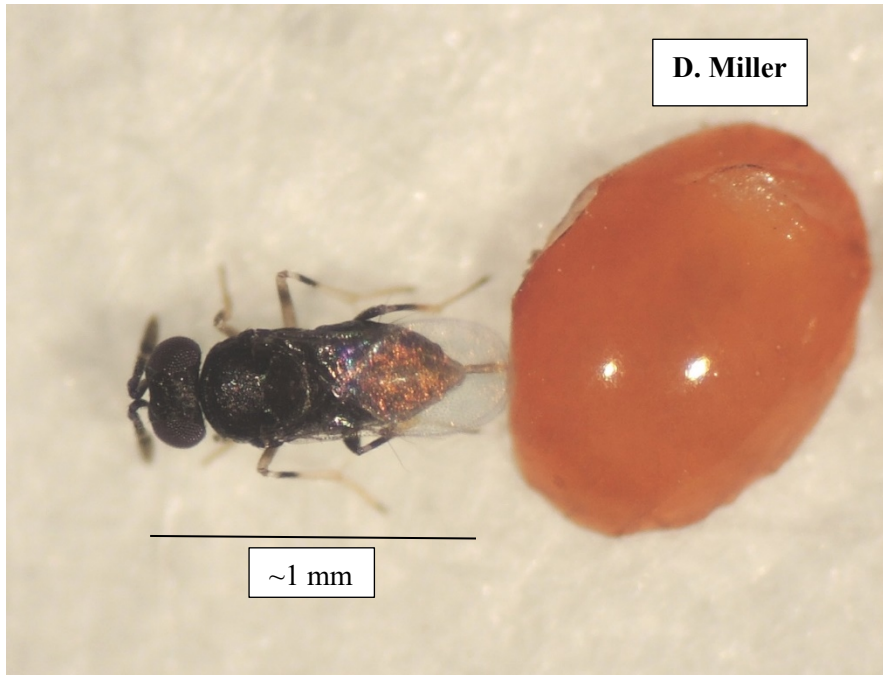
Tetrastichus planipennis Look-a-likes





***Oobius agrili* (Encyrtidae)**

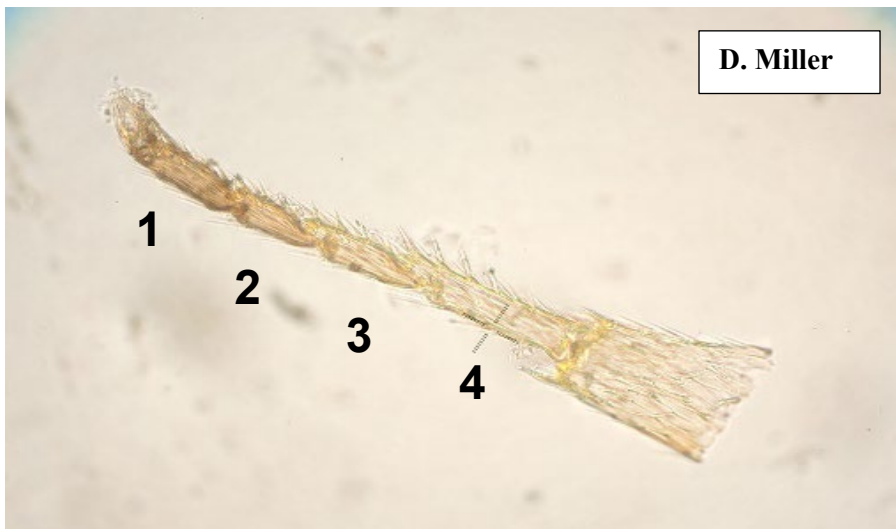
Oobius agrili released for EAB biocontrol are all parthenogenic females, i.e. they do not need to mate and only produce daughters. Adults are very small (~1 mm long), have compact stout bodies with no waist, and a visible, short ovipositor. Their body color is black to dark brown with a blue-green sheen,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



Their antennae are distinctive. The segment (red arrow) before the clubbed clava is clear/yellow, while the other segments are darker (see photo of adult below). The antennae have 9 segments: scape (1), pedicel (2), six funiculi (3-8) and the clava (9) is distinctly clubbed. The antennae are elbowed (or genticulate).

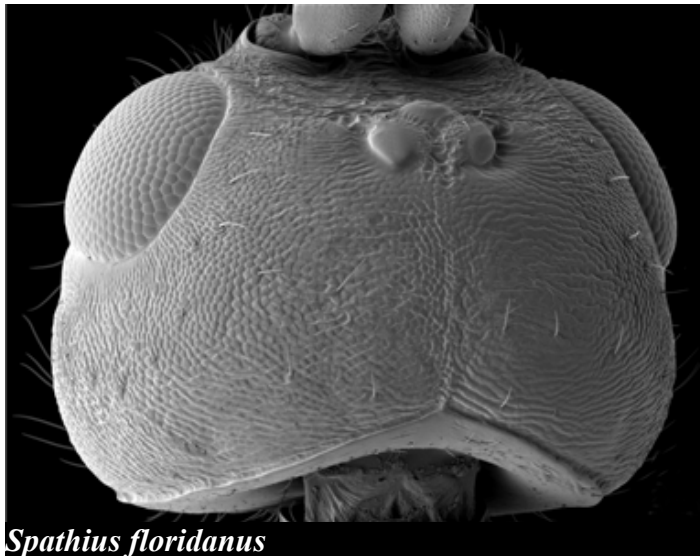


Tarsus of *O. agrili* showing four tarsal segments. All known native species of *Oobius* have five tarsal segments. Note because *Oobius* are so small, you may not be able to see this feature clearly on your microscope.



***Spathius* species (Braconidae)**

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

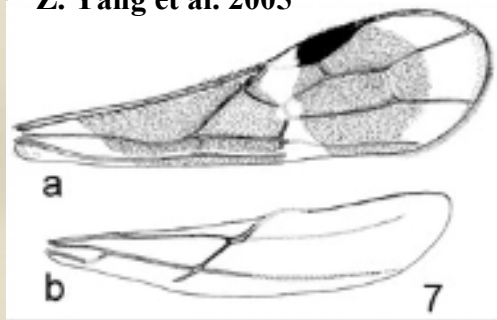


On the left is the head of the native with lots of wrinkles, on the right is *Spathius agrili* which has a totally smooth head. *Spathius galinae* also has a smooth head, similar to *Spathius agrili*.

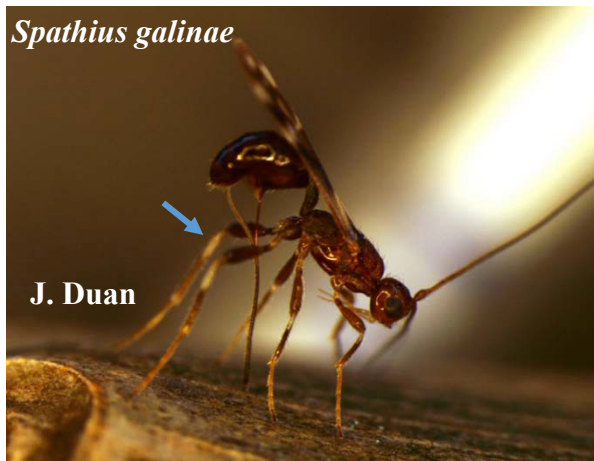


Spathius agrili

Z. Yang et al. 2005



Spathius agrili, like most *Spathius* species, has a distinct clear stripe across the middle of the forewing.



Spathius galinae

J. Duan

Belokobylskij et al. 2012

Spathius galinae



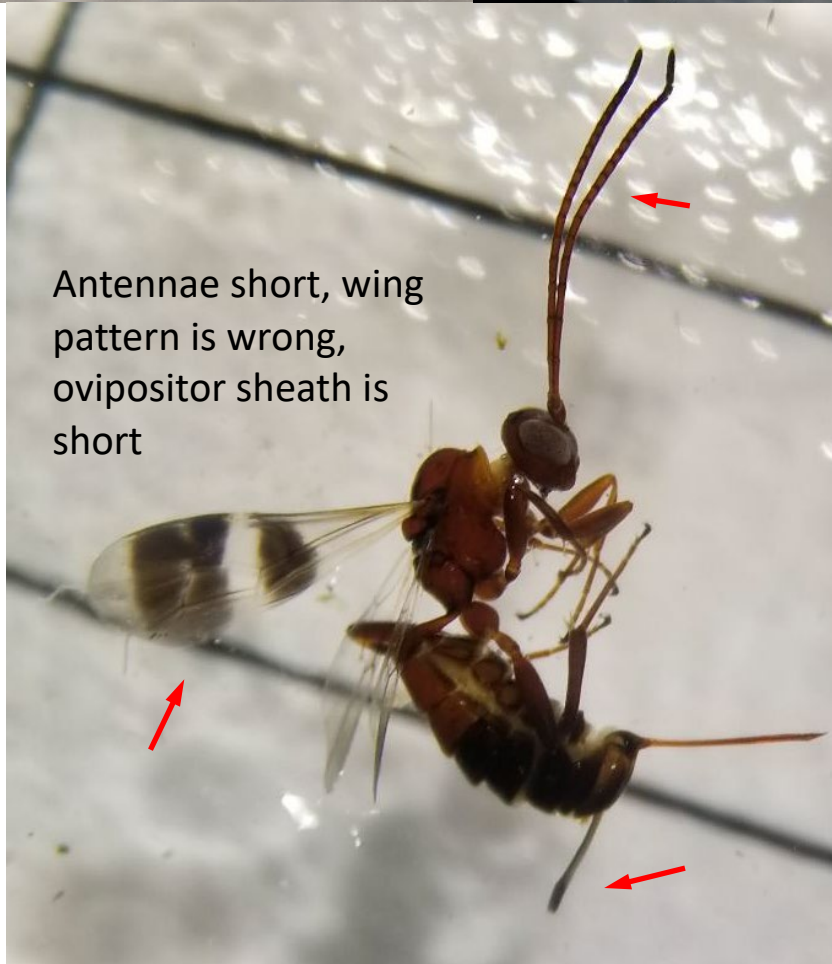
M. Barrett

Spathius galinae



Spathius galinae looks very similar to *S. agrili*. It differs in that the wings look spotted rather than striped, the ovipositor is longer relative to the size of the body, and the base of the tibia of all legs is yellowish (see blue arrow in photo above).

Spathius Look-a-likes



Atanycolus sp.

Atanycolus is a large native parasitoid with a very distinct red or orange abdomen. In contrast, the head and thorax are jet black. The wings are dark grey.

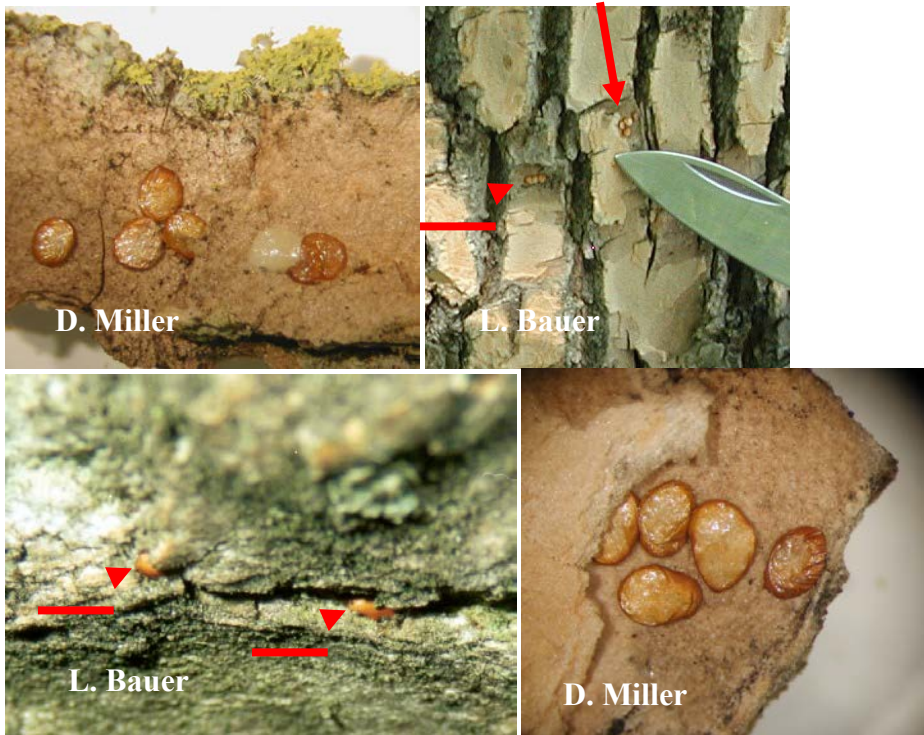


Mention of companies or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

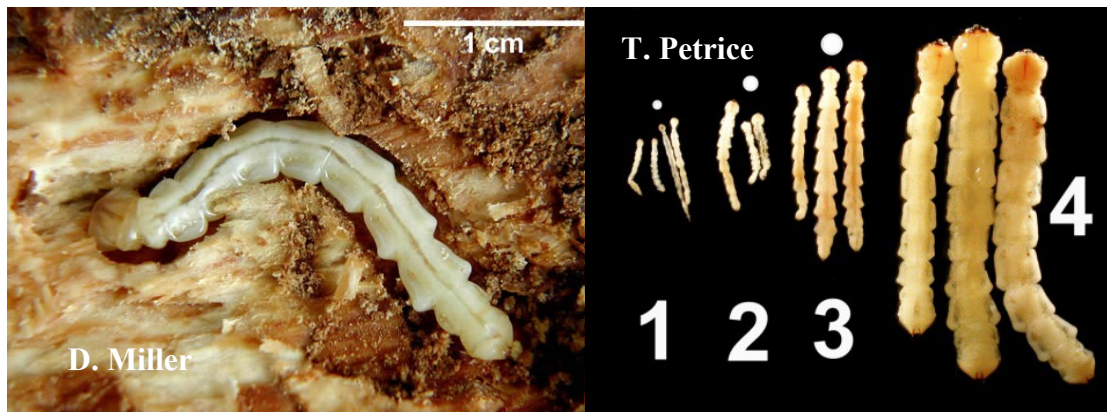
Appendix A – EAB Life Stages and Damage

EAB Life-Stages

EAB eggs (newly laid egg is white maturing to an amber color; clutches of eggs laid between bark flakes; single eggs in bark crevices)



EAB larvae (1-4 indicates the four instars)



Appendix A – EAB Life Stages and Damage

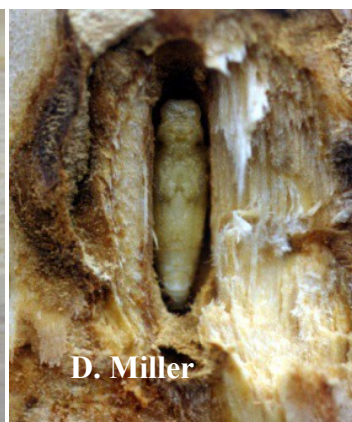
*EAB larva (head is to the left;
note urogomphi at the posterior end of larva)*



EAB Adults

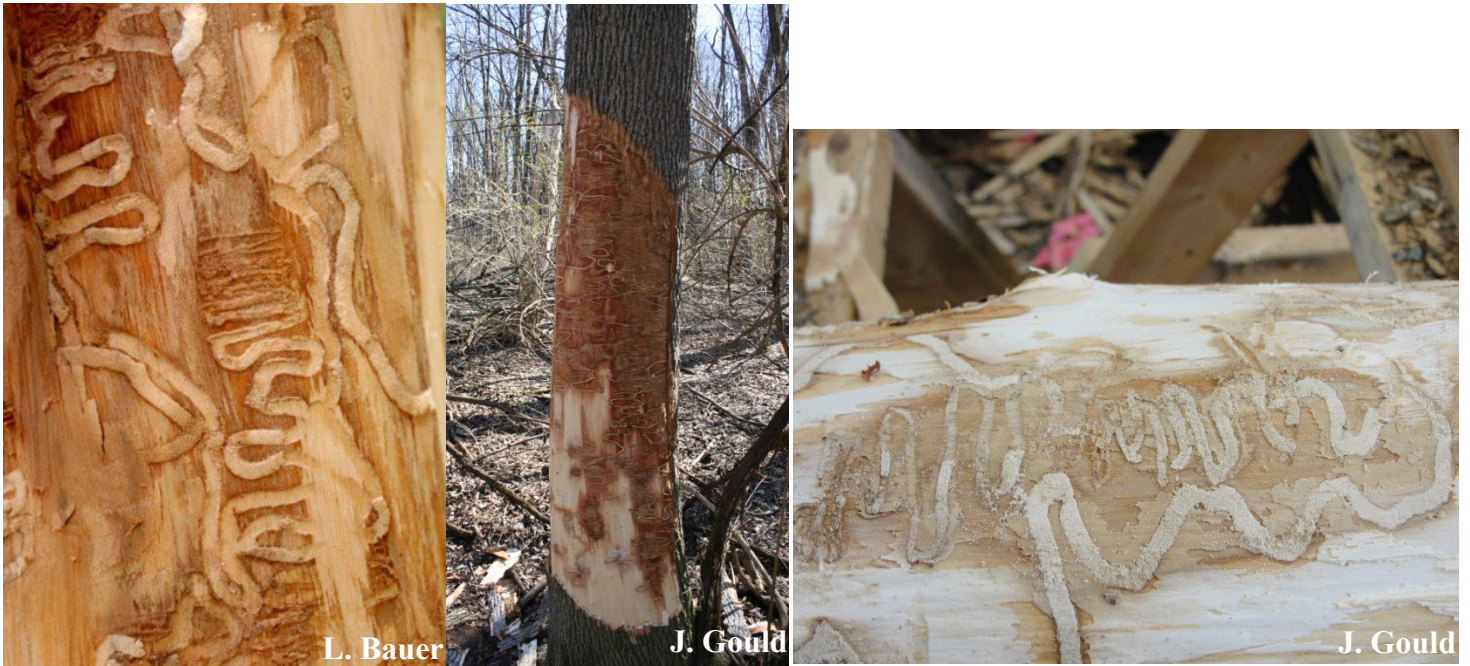


*EAB J-shaped larvae in pupal chamber in outer sapwood (left); pre-pupa (center); pupa (right);
stages of maturing pupae (bottom left)*



Appendix A – EAB Life Stages and Damage

Larval Galleries



External signs of EAB overwintering chamber under the bark. The photo on the left shows the EAB gallery filled with light colored frass and the photo on the right shows the exit portals of three pupal chambers, each with 2 holes filled with frass.



Signs of EAB infestation

Thinning Ash Crowns



Epicormic shoots in winter (left) and summer (right)



Appendix A – EAB Life Stages and Damage

Bark Split with Larval Galleries Beneath the Bark (note callusing around old gallery)



Woodpecker Feeding on EAB in lower ash trunk and midcrown



Appendix A – EAB Life Stages and Damage

Adults emerging from D-shaped exit holes



D-shaped exit holes



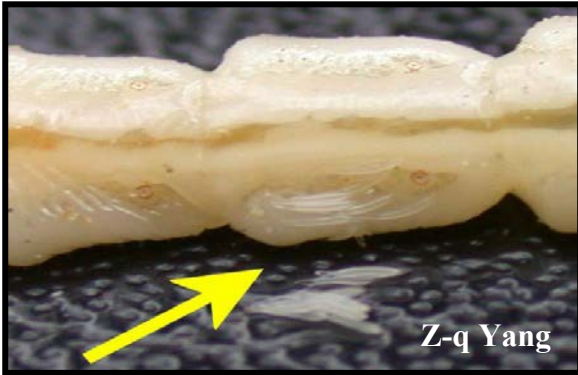
Additional photos and specific morphological and physiological information can be found in the EAB Program Manual at:

http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/emerald_ash_borer_manual.pdf

Life stages of EAB Parasitoids

Spathius agrili

S. agrili lays eggs on the surface of EAB larvae.



Larvae of *S. agrili* feed externally on an EAB larva.



Silken cocoons of *S. agrili* in the host gallery contain mature larvae or pupae.



Appendix B - Parasitoid Life Stages

Female *S. agrili* lay eggs through ash bark onto an EAB larva.



Spathius galinae

Spathius galinae larvae and cocoons



Spathius galinae adult female



Appendix B - Parasitoid Life Stages

Tetrastichus planipennis

Immature T. planipennis larvae inside EAB



Mature *T. planipennis* larvae inside an EAB larva



T. planipennis larvae emerge from host remains and pupate in the gallery.

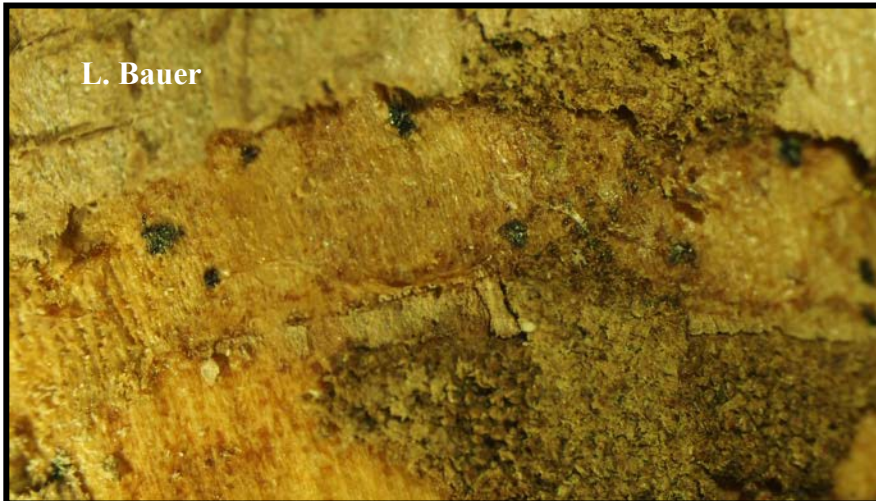


Appendix B - Parasitoid Life Stages

T. planipennisi larvae develop asynchronously, and larvae and pupae are often found together inside one EAB gallery.



T. planipennisi meconia (waste) leave black spots in the empty EAB gallery after adult emergence is complete.

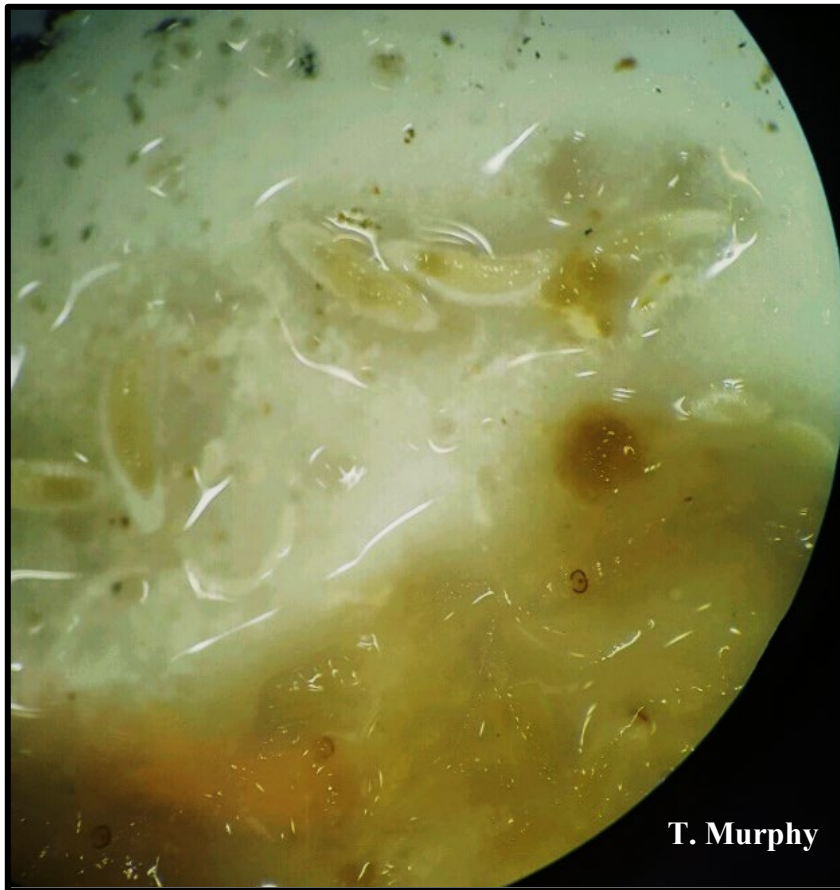


T. planipennisi female lays eggs in an EAB larva through ash tree bark.



Appendix B - Parasitoid Life Stages

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*

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



Appendix B - Parasitoid Life Stages

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. planipennis* larva.



Oobius agrili

EAB eggs often turn dark brown when parasitized by *O. agrili*; unparasitized, healthy eggs remain amber in color (center egg).



Appendix B - Parasitoid Life Stages

Adult *O. agrili* chew a circular hole through the EAB egg shell and emerge.



O. agrili female parasitize EAB eggs laid on ash bark



Appendix C – Crown Condition of Ash Trees

Crown-class condition for ash trees infested with EAB (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)

1



2



3



4



5



Appendix D – Helpful Links

mapBioControl (to enter release and recovery data):

www.mapbiocontrol.org

Growing Degree Days:

<http://uspest.org/US/>

General EAB Information

EAB Program Manual:

http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/emerald_ash_borer_manual.pdf

APHIS Emerald Ash Borer Home Page:

<https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/emerald-ash-borer>

<http://www.emeraldashborer.info/>

<https://www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/the-threat/emerald-ash-borer/emerald-ash-borer-beetle>

EAB Biological Control:

http://nrs.fs.fed.us/disturbance/invasive_species/eab/control_management/biological_control/

Appendix E – Releasing Parasitoids for Optimal Establishment

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 1: 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 <i>T. planipennisi</i> establishment
51-80%	2,985	92%
26-50%	3,500	78%
11-25%	3,975	50%
0-10%	-	23%

Appendix E – Releasing Parasitoids for Optimal Establishment

Figure 1:

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

