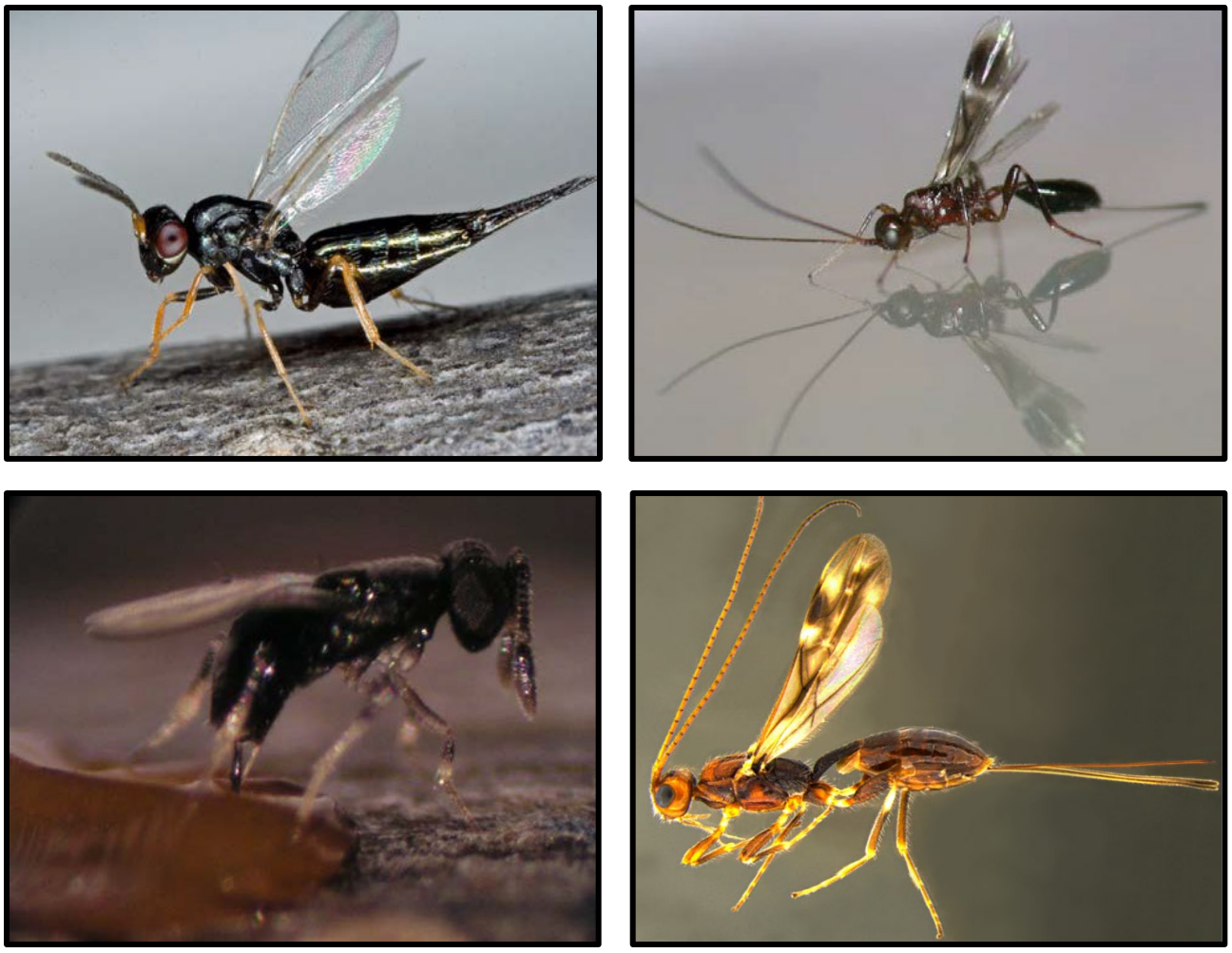
Emerald Ash Borer Biological Control Release and Recovery Guidelines 2026



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Emerald Ash Borer, *Agrilus planipennis* (Fairmaire) Biological Control Release and Recovery Guidelines 2026

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CONTENTS

EAB PROGRAM CONTACTS 1
PARASITOID RELEASES 1
PARASITOID RECOVERY 1
OTHER PROGRAM CONTACTS 2
ADDITIONAL TRAINING RESOURCES 3
RELEASE SITE SUBMISSION PROCESS 4
INTRODUCTION 5
A BRIEF HISTORY OF EAB IN NORTH AMERICA 5
LIFECYCLE OF EAB 6
Adults 6
Eggs 7
Larvae 7
Overwintering larvae, prepupae, and pupae 7
DAMAGE AND SIGNS OF INFESTATION 7
HOST RANGE OF EAB 8
BIOLOGICAL CONTROL (BIOCONTROL) OF EMERALD ASH BORER 9
Biology of EAB Biocontrol Agents 9
Rearing EAB Parasitoids 11
Project Status 11
REFERENCES 13
PARASITOID FIELD RELEASE GUIDELINES 16
OUTLINE OF BIOCONTROL RELEASE SITE SUBMISSION PROCEDURE 16
RELEASE SITE SELECTION 17
General Site Characteristics 18
Minimum Acreage 18
Relative Density of Ash 18
Ash Tree Size Class 18
Density of EAB and Health of Ash Trees 19

Access and Permissions 20
DETAILED SITE SUBMISSION INSTRUCTIONS 21
FIELD RELEASE OF PARASITIODS 23
Release Considerations: Which species, where, and when 23
Tetrastichus planipennisi 24
Spathius galinae 25
Spathius agrili 26
Oobius agrili 26
Requesting Parasitoids 26
Receipt of Parasitoids 27
Care of Parasitoids if Release is Delayed 28
Transporting Parasitoids to Field Sites 28
FIELD RELEASE OF PARASITOIDS 29
Adult Parasitoids in Plastic Cups 29
Larval Parasitoid Pupae in Small Ash Bolts 30
Oobius agrili Pupae inside EAB Eggs in Oobinators 30
ENTER RELEASE DATA INTO MAPBIOCONTROL 31
PARASITOID RECOVERY GUIDELINES 33
RECOVERY OVERVIEW 33
RECOVERY METHOD: YELLOW PAN TRAPS (YPTs) 35
Yellow Pan Trap Deployment Considerations 35
Assembling and Deploying Yellow Pan Traps 38
Servicing Yellow Pan Traps 39
Processing Yellow Pan Trap Samples 41
Overview of Identifying EAB Parasitoids in YPT Samples 44
RECOVERY METHOD: TREE PEELING AND DEBARKING 48
Sampling for Larval Parasitoids (*T. planipennisi* and *Spathius* spp.) 48
Felling and Peeling Logs to Recover Larval Parasitoids 48
Peeling Standing Trees for Larval Parasitoids 52

Sampling for Egg Parasitoid (*Oobius agrili*) 53
Choosing Promising Trees 53
Bark Scraping Cut Logs 53
Bark Scraping Live Trees 54
Processing Bark Samples 56
Rearing Bark Samples 56
Sifting Bark Samples 57
RECOVERY METHOD: BARREL EMERGING FELLED LOGS OR BRANCHES* 59
Choosing Promising Trees 59
How to Build Barrels 59
Storage Conditions 61
Labeling and Monitoring 61
Collecting Parasitoids 61
ENTER RECOVERY DATA INTO MAPBIOCONTROL 62
Identification Information 62
Location Information 62
Sampling Information 62
PUBLICATIONS ON RECOVERY METHODS 64

List of Appendices:

Appendix A – EAB life stages and damage

Appendix B – Parasitoid life stages

Appendix C – Parasitoid identification check lists

Appendix D – Releasing parasitoids for optimal establishment

Appendix E – Crown condition of ash trees

Appendix F – Native and naturalized parasitoids

Appendix G – Helpful links

EAB PROGRAM CONTACTS

PARASITOID RELEASES

USDA APHIS PPQ EAB Biocontrol Rearing Facility

5936 Ford Ct, Ste. 200

Brighton, MI 48116

Email: EAB.Biocontrol.Program@usda.gov

Lab Number: 810-844-2711

The deadline to submit site requests for program parasitoid releases is **December 1**.

Scott A. Whitehead, Release Coordinator

Email: scott.a.whitehead@usda.gov

Elisabeth Darling, Supervisory Entomologist

Email: elisabeth.darling@usda.gov

PARASITOID RECOVERY

Send adult specimens from yellow pan traps to:

Andrea Anulewicz
5936 Ford Ct, Ste. 200
Brighton, MI 48116
Email: andrea.anulewicz@usda.gov

Send EAB eggs/*Oobius agrili* from bark sifting or bark rearing to:

Toby Petrice
2601 Coolidge Rd, Ste. 203
East Lansing, MI 48823
Email: toby.petrice@usda.gov

If you have larval/cocoon specimens from tree peeling, certain specimens can be identified morphologically while others need to be DNA sequenced. Please email Theresa Booth (theresa.c.booth@usda.gov) for where to send them for identification.

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ADDITIONAL TRAINING RESOURCES

Every two years, the program hosts an in-person EAB Biocontrol Release and Recovery Workshop for interested program participants, including representation from federal, state, university, and tribal affiliates. Contact Russ Bulluck (russ.bulluck@usda.gov) for more information.

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

Module 1 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 7, 2022 - YouTube

Introduction, background, and lifecycle of EAB, how to identify EAB and possible look-a-likes, history of biological control of EAB, how to identify ash and possible look-a-likes, impacts of EAB parasitoids. **(1 hour 40 minutes)**

Module 2 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 14, 2022 - YouTube

Mass rearing of EAB biological control agents for release, choosing sites and considerations for releasing parasitoids, conducting parasitoid releases, and an introduction to MapBioControl. **(1 hour 47 minutes)**

Module 3 Emerald Ash Borer Release and Recovery Guidelines – April 21, 2022 - YouTube

Evaluating parasitoid establishment: debarking for *Oobius agrili*, peeling trees for larval parasitoids, and yellow pan traps. **(2 hours 21 minutes)**

RELEASE SITE SUBMISSION PROCESS

PLEASE NOTE: If your site is selected for biocontrol releases, you are committing to <u>four consecutive years</u> of program participation. You will be expected to do two years of releases, followed by two years of recovery work.

Step 1: Create a MapBioControl.org account.

Step 2: Locate a potential release site and record the following information:

<u>Site Basics</u>

1. Status (Proposed)
2. State
3. Date
4. Site Name
5. Site Location
6. Latitude and longitude (dd.dddddd)
7. Type (Program or Research)

<u>General Details</u>

1. Site size (acres)
2. Percent of ash present
3. Top three dominant tree species
4. EAB density (low, medium, or high)

Other physical details are optional.

Step 3: Enter this information into MapBioControl and record your new site ID.

Step 4: Email the program contacts (EAB.Biocontrol.Program@usda.gov) with your new site ID. They will provide you with a spreadsheet requesting your contact and address information if your site is selected for releases. **Fill this out and return it by December 1 of the year before you intend to release.**

Step 5: Apply for any required local land use permits.

Step 6: Site selections will typically be released to cooperators in early March. **Please check to see if your site made the selected list and reply to confirm you will be conducting releases!**

<u>FAQs</u>

1. ***How are site selections determined?***
   The Rearing Facility Release Coordinator and Supervisory Entomologist work together to determine which sites have the highest priority for releases that year. Sites are given higher priority if they are located in counties or states where releases have not been conducted yet.

2. ***When do releases typically begin?***
   Parasitoid release season is typically from April to September. Release dates are calculated based on growing degree days (GDD50F), so dates vary greatly by location and parasitoid species.

3. ***My site wasn't selected for releases. Can I be considered in the future?***
   Absolutely! Each year we do our best to try and accommodate as many sites as production allows. You are welcome to resubmit the site the following year if it was not selected.

4. ***Do I need a permit to receive parasitoids?***
   The Rearing Facility holds permits to ship to all EAB-infested states in the U.S., so you will not need a permit to receive them. A copy of this permit will be included inside of your shipment. However, you will need to ensure that you have the proper permits or land use approvals for local releases.

5. ***Can I release on my private land?***
   No, we do not accommodate releases on private land. Releases must be conducted on federal, state, county, tribal, or other public lands.

INTRODUCTION

A BRIEF HISTORY OF EAB IN NORTH AMERICA

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

Shortly after EAB was discovered in North America, federal and state regulatory agencies placed infested counties under quarantine and initiated eradication activities. 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. EAB was federally deregulated in FY2021.

As of March 2026, EAB infestations in the U.S. were known in Alabama, Arkansas, Colorado, Connecticut, Delaware, District of Columbia, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, and Wisconsin and the Canadian provinces of British Columbia, Manitoba, New Brunswick, Nova Scotia, Ontario, and Québec.

The continued spread of EAB threatens our ash resources and will permanently alter forest ecosystems in North America. Ash wood is used for a variety of applications including tool handles, baseball bats, furniture, cabinetry, solid wood packing materials, pulp, and paper. Black ash is economically and culturally significant to many tribal nations in the Northeast and Great Lakes regions for its use in traditional basketry. In addition to its value

to the timber industry and the forest ecosystem, ash was a popular landscape tree and the spread of EAB has urban, suburban, and nursery industry impacts. The PPQ EAB program aims to maintain ash as a viable part of the American landscape by managing known infestations with a robust biological control program, while also incorporating other management methods and outreach to minimize EAB's impacts.

The **USDA APHIS PPQ EAB Biological Control Rearing Facility** (hereafter referred to as the **Rearing Facility**) opened in Brighton, Michigan in 2009. The Rearing Facility mass rears four species of EAB parasitoid for program releases across the United States and occasionally in Canada. Since its establishment, the Rearing Facility has produced over 9 million parasitoids that have been released in 34 of 37 EAB-infested states and Washington, D.C.

LIFECYCLE OF EAB

EAB takes one or two years to complete its lifecycle depending on temperature, latitude, altitude, local population density, and tree health. Below is a description of the EAB lifecycle. Development estimates are reported as growing degree days (GDD) base 50°F. GDD accumulations and forecasts for EAB can be found at https://uspest.org/cgi-bin/ddmodel.us.

Adults

EAB adults begin to emerge from ash trees after the accumulation of ~400 GDD50F. Peak adult activity occurs at ~1,000 GDD50F. To emerge from ash trees, adults chew D-shaped exit holes (Appendix A) through the bark and are immediately capable of flight upon emergence. 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 laying eggs 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 x 0.6 mm and creamy white when laid; fertile eggs gradually turn amber after a few days (Appendix A). The eggs hatch after 2-3 weeks.

Larvae

Newly hatched larvae bore through the bark to the cambial region where they feed until mature. There are four stages (instars) of larval development (Appendix A). As they feed, the larvae create serpentine galleries filled with frass (excrement), which enlarge in width as the larvae grow (Appendix A). Larvae are creamy white and dorsoventrally flattened (Appendix A). When fully mature, 4th-instar larvae are 26-32 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, and pupae

In the fall, mature 4th-instar EAB larvae excavate pupal chambers in the new sapwood or outer bark where they fold into overwintering “J-larvae” (Appendix A). In winter and spring, the J-larvae shorten into prepupae and then shed their cuticle to become pupae. Pupae are initially creamy white, but the eyes turn red and the body darkens as they develop (Appendix A). EAB larvae that are immature as cold weather arrives in the fall will overwinter in their larval feeding gallery. J-larvae will pupate and complete development (i.e. become an adult beetle) in the spring, while younger larvae may require another summer of feeding before pupating and completing development the following spring.

DAMAGE AND SIGNS OF INFESTATION

EAB larvae damage ash trees by feeding on the phloem and cambium. In a new infestation, when just a few EAB larvae infest a tree, the tree responds by forming a callus (scar tissue)

around EAB galleries, and the tree may show few outward signs of infestation. On some trees or branches, the callus may cause the bark to split open, exposing the EAB gallery beneath (Appendix A). Although difficult to detect, especially high in the canopy, the D-shaped exit holes chewed by emerging adults are diagnostic indicators of EAB infestation (Appendix A).

As EAB larval population density increases, the movement of nutrients through the phloem is disrupted and increases evidence of tree stress, 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. Symptoms of woodpecker feeding include the removal of bark flakes, also called bark scaling or "blonding" due to the exposed bark being lighter in color, and feeding holes through the bark (Appendix A).

HOST RANGE OF EAB

In North America, EAB attacks ash species in the genus *Fraxinus*, including but not limited to green ash (*F. pennsylvanica*), white ash (*F. americana*), black (brown) ash (*F. nigra*), pumpkin ash, (*F. profunda*), blue ash (*F. quadrangulata*), and Oregon ash (*F. latifolia*). Sixteen native species of ash, some with limited distributions in North America, are now threatened by EAB. In China, native ash species, including Chinese ash (*F. chinensis*) and Manchurian ash (*F. mandshurica*), are less susceptible to EAB than North American species commonly planted in China such as green ash (*F. pennsylvanica*) and velvet ash (*F. velutina*). In 2014, EAB was observed attacking North American white fringetree (*Chionanthus virginicus L.*) in Dayton, Ohio [1]. It was later found that EAB can attack and develop on cultivated olive (*Olea europaea L.*), a close relative of white fringetree [2]. However, the impact of EAB on white fringetree and cultivated olive is not well understood.

BIOLOGICAL CONTROL (BIOCONTROL) OF EMERALD ASH BORER

Classical biocontrol is the practice of importing and releasing natural enemies from a pest's native range to control target pest populations in its invasive range. Biocontrol has been used for over 100 years in the U.S. It has successfully controlled invasive plant and insect pests such as spongy moth, winter moth, ash whitefly, eucalyptus longhorned borer, purple loosestrife, and Klamath weed. Foreign exploration for natural enemies of EAB in its native range in northeast Asia began in 2003. In Asia, EAB population densities are typically low. This is because of EAB resistance in Asian ash species, ash scarcity and patchiness of forests, and the coevolved complex of natural enemies. Foreign explorations in China, Russia, and South Korea have yielded several hymenopteran parasitoids of EAB. Four species are approved for release in the contiguous United States as biocontrol agents for EAB.

Biology of EAB Biocontrol Agents

Oobius agrili is a solitary egg parasitoid of EAB collected from China [3]. It was found to parasitize up to 60% of EAB eggs laid during the summer in some areas of China. Tiny female *O. agrili* accomplish this by searching the bark of ash trees for EAB eggs. When *O. agrili* finds an EAB egg, it injects its own egg inside (Appendix B) where the larva will hatch, grow, and kill the host egg. Each *O. agrili* adult can parasitize up to ~60 EAB eggs during its lifetime. *Oobius agrili* overwinter as larvae inside of EAB eggs and emerge as adults the following spring, repeating the cycle for at least two generations during the EAB egg-laying season. All *O. agrili* being released are females that reproduce without mating to produce only daughters.

Tetrastichus planipennisi is a gregarious larval parasitoid of EAB collected from China [4]. *T. planipennisi* is an endoparasitoid that lives and develops inside an EAB larva, eventually emerging and killing its host. Female *T. planipennisi* parasitize EAB by drilling into the bark with their ovipositor and laying eggs inside a larva. One EAB larva can produce over 100 *T. planipennisi* adults. *Tetrastichus planipennisi* completes several generations each year, overwintering as larvae inside their host or as prepupae in the host gallery (Appendix B). As the weather warms in spring, the overwintering larvae pupate and complete their

development, chewing small round exit holes through bark above the gallery and emerging to seek EAB larvae to parasitize. Due to the short ovipositor of *T. planipennisi*, they are more successful at parasitizing EAB larvae in small diameter ash trees and branches up to 11 cm in diameter at breast height (DBH) [5]. Research has shown that for *T. planipennisi* populations to persist, suitably sized (3rd or 4th instar) EAB larvae need to be readily available when the parasitoids emerge in the spring. Because of this, *T. planipennisi* is now preferentially released in areas where at least 25% of EAB have a two-year life cycle and where modelling predicts the parasitoid will establish [6] (Appendix D).

Spathius agrili is a gregarious larval parasitoid of EAB collected from China [7]. It was found to parasitize up to 90% of EAB larvae in ash trees in Tianjin, China, where the climate is relatively mild and EAB has a one-year life cycle. Releases of *S. agrili* are limited to EAB infestations in the southern United States where at least 50% of EAB have a one-year life cycle. Modeling predicts this will be in areas that accumulate more than 3,500 GDD50F (Appendix D). *Spathius agrili* is an ectoparasitoid that lives externally on EAB larvae. Female *S. agrili* parasitize EAB larvae by drilling through the bark with their ovipositor (Appendix B) and laying an average of 8 eggs on the outside of its host. The hatching parasitoid larvae (Appendix B) feed and develop on the EAB larva, eventually killing it. The cycle is repeated 1-2 times each year depending on climate. *Spathius agrili* overwinter as larvae or pupae in the host gallery. Mature larvae spin silken cocoons in which they pupate and emerge as adults during the following summer.

Spathius galinae has a biology similar to that of *S. agrili*, but *S. galinae* originated in the Russian Far East [8]. This region is a better climate match to northern regions of North America than the part of China where *S. agrili* was collected, and unlike *S. agrili*, *S. galinae* has established well in the northern United States. In addition, both *S. galinae* and *T. planipennisi* are better suited to establish in northern regions due to the availability of late-instar EAB larvae in early spring when adult parasitoids emerge. For these reasons, *S. galinae* is preferentially released in areas where at least 25% of EAB have a two-year life cycle (see Appendix D). *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), which includes about 95% of all ash in the northeastern United States [9].

Rearing EAB Parasitoids

The Rearing Facility produces thousands of EAB parasitoids each year for field releases. These small parasitic wasps must be reared in EAB eggs or larvae, which are produced or harvested from ash trees felled and removed from EAB-infested woodlots. Although the parasitoids are reared and stockpiled throughout the year for release during the field season, the rearing methods are both time and labor intensive. Research to develop an artificial diet for EAB is ongoing, but for now fresh ash logs and leaves are needed for production of EAB and its parasitoids. The egg parasitoid, *Oobius agrili,* is reared in EAB eggs laid on paper strips by EAB adults. The three larval parasitoids, *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.

Project Status

EAB biocontrol was initiated more than 20 years ago. During those early years, a dedicated group of federal, university, and state scientists completed all the steps necessary for implementation of a successful classical biocontrol project. They conducted host specificity testing [10-13]; applied for and received parasitoid release permits; developed methods for mass rearing [14]; and improved release and recovery methodology [15].

Our work continues today by expanding releases to new areas, evaluating program success through parasitoid monitoring and impact studies, and using these results to optimize protocols for different regions of the country. A nationwide consortium of cooperators collects data showing where the various species of parasitoids have established. Since 2009, the Rearing Facility has produced and released more than 9 million parasitoids in 34 states (Arkansas, Colorado, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin), the District of Columbia, and five Canadian Provinces (Manitoba, New Brunswick, Nova Scotia, Ontario, and Québec).

The first step in evaluating the efficacy of a biocontrol program is to determine if and where parasitoids are being recovered. *Oobius agrili* has been recovered in 19 states, *Spathius agrili* in 15 states, *Spathius galinae* in 18 states, and *Tetrastichus planipennisi* in 21 states. However, just because a parasitoid has been recovered does not mean it is established. Parasitoids are only considered established in an area if they are recovered at least two years after the last releases have occurred and continue to be recovered in following years. For instance, although *Spathius agrili* can overwinter in northern climates and was recovered in multiple states, it did not establish in any northern states and so far only shows evidence of establishment in Tennessee. Research suggests that *S. agrili* is not well synchronized with EAB phenology in northern climates [16]. After *S. agrili* was found to not be establishing, explorations began to find another large parasitoid to control EAB in larger ash trees and complement *T. planipennisi*, which has a short ovipositor and cannot reach EAB larvae in trees or branches larger than 11 cm DBH [5]. This is when *Spathius galinae* was discovered from Vladivostok, Russia, and climate matching indicated it should do well in the northern United States. Since 2015 when it was approved for release, *S. galinae* has established in many northern states, along with *T. planipennisi* and *O. agrili*.

Another important consideration when evaluating the success of a biocontrol program is whether the parasitoids are capable of dispersing away from the release point without human intervention. Several studies have investigated dispersal of the larval parasitoids. Scientists found that *T. planipennisi* was highly capable of following EAB populations as they moved, and that this parasitoid can disperse over 10 km in 3 years [17]. *Spathius galinae* is also a good flier and can disperse over 14 km in 5 years [18]. In Maryland, *S. galinae* was found 90 km away from the nearest release site within 3 years [19]. *Oobius agrili* disperses more slowly due to its tiny size, and was found 1-3 km away from release sites within a few years [20].

Scientists have been studying the impact of parasitoids on EAB population density and have documented an increase in parasitism following release while densities of EAB declined. However, when thousands of parasitoids are released in a forest with millions of EAB, especially in the early years of the program when the larger parasitoid *Spathius agrili* failed to establish, most mature ash trees died. For ash to remain a viable component of these forests, it is crucial that the parasitoids can control EAB in regenerating ash. Several

studies in Michigan and New York show that *T. planipennisi* plays a considerable role in reducing the net reproductive rate of EAB in small, regenerating ash and aftermath forests [21-23]. Recent studies show that the parasitoid *S. galinae* is also having a significant impact on EAB populations [22, 24-26].

Recovery of ash populations will occur on the order of decades rather than years. However, we are beginning to see signs of ash recovery. Recent assessments of ash regeneration at long term study sites in New York demonstrate that the density of seedling ash at biocontrol release sites did not significantly decline between 2014 and 2021, and the density of larger saplings (>1m) significantly increased [27]. Studies in Michigan also documented high ash regeneration in post-invasion forests, despite high mortality of large diameter trees [28, 29]. Between 2012 and 2015, ash mortality was reduced in biocontrol release plots, ash diameter increased, and ash regeneration, while variable between sites, was generally higher in release plots than control plots that did not receive biocontrol agents [29]. Scientists will continue to document the impacts of the EAB biocontrol program on pest density and ash health.

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PARASITOID FIELD RELEASE GUIDELINES

PLEASE NOTE: If your site is selected for biocontrol releases, you are committing to four consecutive years of program participation. You will be expected to perform two years of releases, followed by two years of recovery efforts.

Program Site Release and Recovery Timeline
Years 1 and 2: Perform parasitoid releases.
Years 3 and 4: Perform parasitoid recovery efforts.

OUTLINE OF BIOCONTROL RELEASE SITE SUBMISSION PROCEDURE

1. **Select Release Site(s)** in an area with good access, high density of ash trees of various sizes (seedlings, saplings, and mature trees), and mild to moderate EAB infestation.
2. **Create a MapBioControl Account and Enter Site Data** 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. Record MapBioControl ID number for each site created.
3. **Add General Site Details and Physical Characteristics.** This information is useful for Rearing Facility personnel to prioritize and select the best site(s) for parasitoid release. Enter this information manually on the MapBioControl web site (www.mapbiocontrol.org).
4. **Notify the EAB Biocontrol Program** (EAB.Biocontrol.Program@usda.gov) that your site has been submitted to MapBioControl. The program will send you an excel spreadsheet to provide the MapBioControl ID number for your site, desired shipping address, and contact information. **All site requests must be submitted by December 1 in the year before you plan to release parasitoids.** If you want to release parasitoids in 2027, your request is due by December 1, 2026.
5. **Obtain Local Land Use Permits.** Certain locations such as state parks, national forest land, or wildlife refuges may have specific local permit requirements for releasing and/or recovering parasitoids on their land. Be sure to check the regulations applicable to your site.
6. **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. Enter

Release data into MapBioControl using ArcGIS Field Maps on your phone or tablet or online within 48 hours of release. The program will send out a reminder at the end of the season to upload any releases that have not been entered.

7. **Synchronize Your Phone to Collect Data Offline.** If you collect data in offline mode with ArcGIS Field Maps, make sure to synchronize your data once you are back on Wi-Fi to prevent the loss of data. Before you can collect data offline, you will need to download an 'offline area' in Field Maps prior to heading out into the field. Data can also be entered manually.
8. **Assess Parasitoid Establishment.** After completing releases for two consecutive years, your site will be moved into Recovery status. Please see Part C to learn more about recovery guidelines.

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 will be released at sites where they have the highest probability of establishment (Appendix D). The site information described below should be collected and entered into MapBioControl for each site submission. This will help researchers and the Rearing Facility team determine which sites are most suitable for release and establishment of parasitoids.

The guidelines below outline best practices for establishment. Your site may not meet all the criteria, but may still be appropriate for releases if it has compensating strengths or is the most suitable release site for your county. For example, the site may have low acreage, but it has connectivity to other ash plots. If determining the suitability for a particular site is not straightforward, please email the Rearing Facility (EAB.Biocontrol.Program@usda.gov) to note that you have found what you believe to be the most suitable release site for your county, even if it doesn't meet all the requirements below.

General Site Characteristics

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

Minimum Acreage

Wooded areas at least 40 acres in size are preferred. Smaller release sites (<40 acres) will require higher ash densities and/or 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 is ideal. The percentage of ash can be estimated as ≤25%, 26-50%, 51-75%, or 76-100%.

Ash Tree Size Class

Ideally, 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 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 planipennisi*, which has a short ovipositor, appears most likely to establish in areas with some smaller, thin-barked ash trees, where EAB larvae are more accessible.

Density of EAB and Health of Ash Trees

Potential release sites should have low to moderate EAB population densities. Unless there are many young trees in the vicinity, stands with several dead and dying ash trees are not appropriate as release sites because ash and EAB may crash before the parasitoids become established. The only exception is for **aftermath (or post-invasion) forests,** where EAB has been established for a significant amount of time and caused extensive ash mortality, and which is now experiencing ample ash regeneration and new growth. These include counties in the northern U.S. where *Spathius agrili* did not establish which are now eligible for release of *Spathius galinae*, or counties where most of the mature trees have already died but where no parasitoid releases were done previously. Aftermath sites must have plenty of live, small ash trees available for EAB and its parasitoids for establishment to occur. Basal sprouts and saplings need to be over 1 inch in diameter to support EAB.

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 (blonding) (Appendix A). As EAB densities increase, EAB symptoms that are 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 several D-shaped exit holes (Appendix A).

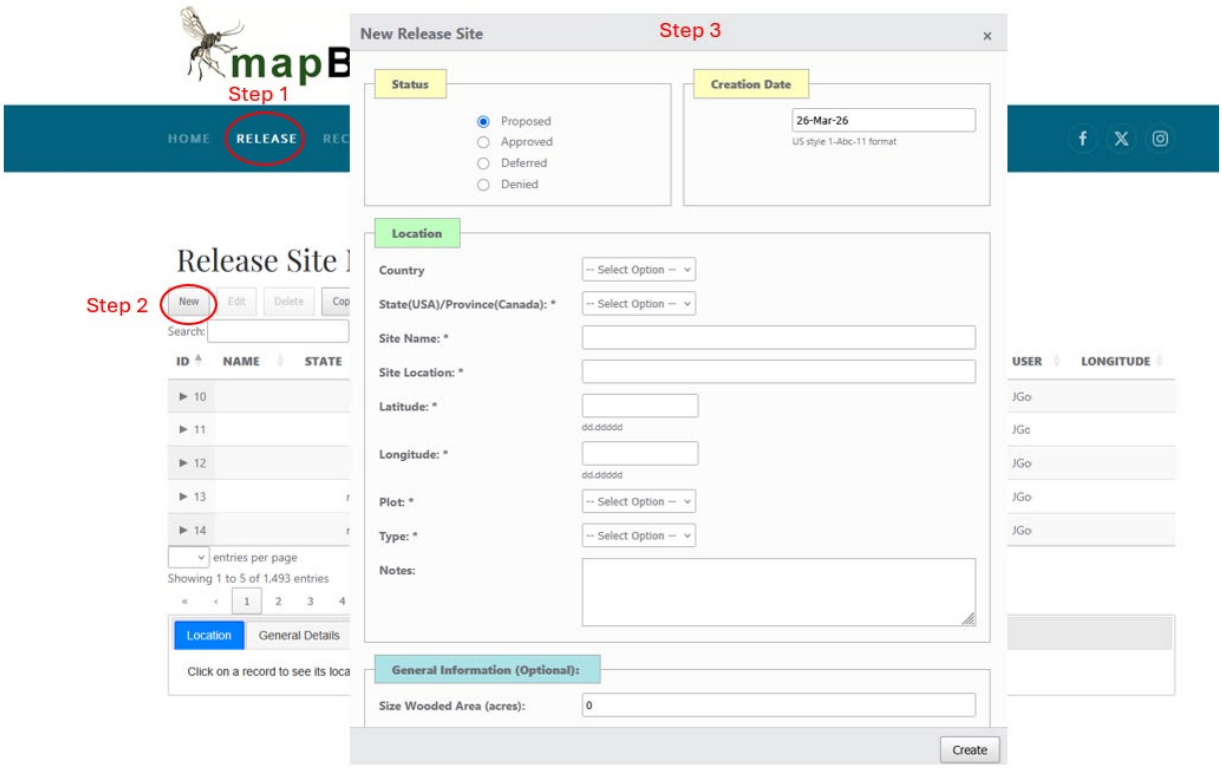
During the spring and summer when the trees have leaves, ash health can be assessed by crown class rankings (Appendix E). Suitable release sites should have mostly healthy trees with an average crown condition of 1 to 2 and only a few trees in condition classes of 4 to 5 (dying or dead). Other insects 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 symptoms of 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 peeling ash trees in the stand. This is a direct estimate of EAB density, but is labor intensive, destructive, and

counter-productive in areas where EAB density is low. Therefore, after EAB has been confirmed in the area, it is okay to use an indirect EAB-density estimate based on the signs and symptoms of EAB infestation in the ash trees.

Access and Permissions

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

DETAILED SITE SUBMISSION INSTRUCTIONS



To submit a new **Release Site** into MapBioControl, click on “Release” in the main banner (Step 1). Click the “New” button in the upper gray table (Step 2), then enter your site data (Step 3 – see below for required fields).

IMPORTANT: The fields denoted with an asterisk are required by the website. The other fields are optional, except for **Ash %** and **EAB Density**, which are **required by the Rearing Facility** to review potential release sites.

For each release site, please enter the following information:

- **Status:** Select "Proposed" because the site has not yet been approved.
- **Creation Date:** Auto-populated but can be modified.

Location

- Country
- **State(USA)/Province(Canada): ***
- **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. Use for research studies.)
- **Type*** (program or research)
- **Notes:** Anything else you want to mention about your site

General Information (Optional)

- **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%)
- **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

- **Dominant Tree Species**
- **2nd most Dominant Tree Species** (if applicable)
- **3rd most Dominant Tree Species** (if applicable)

Physical Details (Optional)

- 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)
- Pair Site (Used for research studies if pairing release and control plots)

After your site submission is generated, you will see it listed as in the screenshot below. **The ID is your Site ID #.** You must keep track of this number as you conduct your releases and recoveries as this number is used by the Rearing Facility when managing all release sites. If you click on your site submission, it will highlight in blue and you can click on the 'Edit' tab to fill in or modify information.

FIELD RELEASE OF PARASITIODS

Release Considerations: Which species, where, and 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 each parasitoid varies with geographic area, and they are not all suitable for releases in all states. We also now have a fourth parasitoid, *S. galinae*, which is suitable for releases in certain areas. Thus, we have made new recommendations based on current research results (Appendix D).

Although higher numbers and frequent releases of parasitoids improve establishment, actual release quantities depend on the Rearing Facility's weekly production capabilities. Minimum recommended numbers for releases are listed below by species, but shipments may vary based on weekly availability and the number of active release sites. When extra parasitoids are available, they are added to the shipment. 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 to increase the likelihood of parasitoid establishment.

Larval parasitoids should be released when late-instar EAB larvae (3rd- and 4th-instar) are present in the field. Firm recommendations on release timing are difficult because EAB larval development is variable and depends on factors such as when the eggs were laid, temperature, and ash tree health. *Oobius agrili* should be released when EAB eggs are present. However, EAB eggs are very small and nearly impossible to find in the field, and EAB larvae are under ash bark and are not accessible without peeling trees. Spreading releases out over multiple weeks helps ensure that the proper stages of EAB eggs and larvae are present for parasitism. Growing degree days (GDD) base 50°F are used to estimate EAB larval development and help the Rearing Facility to decide when to start releases in each state. GDD accumulations and forecasts can be found at http://uspest.org/US/ and http://uspest.org/cgi-bin/ddmodel.us.

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 are available for parasitism in the spring, which occurs when the EAB overwinter as larvae (close to the surface of the tree) rather than as J-larvae (in pupal chambers deeper in the wood). We use a model that delineates the areas expected to have over 25% EAB overwintering as larvae shown in Appendix D.

- If your site accumulates > 3,500 GDD50F in the summer and you would still like to release *T. planipennisi*, please contact the Rearing Facility. You will need to confirm that you have late-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 GDD50F accumulate in the summer, and we do not recommend releasing this species in these locations.
- *Tetrastichus planipennisi* has a short ovipositor. Make sure there are plenty of trees and branches >1 and <5 inches in diameter at your site that contain EAB larvae before releasing.
- First releases will occur 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 there aren't mature larvae in your area in the spring, do not release *T. planipennisi* in the fall; there will be nothing for them to parasitize when they emerge the following spring.
- Release at least 1,200 total females per season.

Spathius galinae

- Counties in the northern U.S. where parasitoids were released but *S. agrili* did not establish are eligible for release of *S. galinae*.
- Aftermath forest sites where the large ash trees have died are still suitable if there are plenty of live small ash trees > 1 inch diameter available for EAB and its parasitoids for establishment to occur.
- *Spathius galinae* also emerges early in the spring and will only be released following the same guidelines as for *T. planipennisi*.
- First releases will occur in the spring after 300 GDD50F have accumulated.
- Releases begin again in the late summer-fall when mature larvae are available (generally between 1400 and 2500 GDD50F).
- If you do not have any mature larvae in the spring, do not release *S. galinae* in the fall; there will be nothing for them to parasitize when they emerge in the spring.
- Release at least 600 total females per season.

Spathius agrili

- *Spathius agrili* will only be released at sites that accumulate more than 3,500 GDD50F. For this reason, we are interested in expanding site releases in more southern states.
- First releases will occur when late-instar EAB larvae are present in ash trees. That could be as early as mid-June in more southern states such as Arkansas, or in mid-July in more central states such as Tennessee. We do not yet have GDD estimates for initiating releases. Peeling bark to quantify EAB larval instars prior to release is recommended.
- Release at least 1,200 females total per season.
- Do not conduct any releases after the end of August.

Oobius agrili

- *Oobius agrili* can be released in all states.
- *Oobius agrili* is released in two phases to maximize the probability of exposure to host eggs. At least 1,200 females should be released each season.
    - Initial releases at 600 GDD50F. Release at least 600 females over 3 weeks.
    - Second round of releases at 1400 GDD50F. Release at least 600 females over 3 weeks.
- Releases can continue until ~2,500 GDD50F have accumulated.

Requesting Parasitoids

Email all parasitoid requests and any questions about site approval or which parasitoids to request to the EAB Biocontrol Program mailbox (EAB.Biocontrol.Program@usda.gov). To be eligible for releases, you must submit your detailed site information to MapBioControl. Doing so will generate an ID number for your site(s). Please record this number and include it in your request.

Receipt of Parasitoids

In the case of unexpected delays in receiving or deploying parasitoids, please contact **Scott Whitehead** (Biological Control Release Coordinator, 810-844-2708) or **Dr. Elisabeth Darling** (Supervisory Entomologist, 248-329-4526), or email the EAB Biocontrol Program mailbox (EAB.Biocontrol.Program@usda.gov).

Parasitoids are shipped by overnight delivery in a cardboard box and should arrive by 10:30AM EST at most locations.

Larval parasitoids will be shipped either as developing pupae inside ash bolts or, less frequently, as adults in 16-oz plastic cups with lids taped on. Honey will be smeared on the lid as a source of food for the adult parasitoids in cups. *Spathius galinae* bolts will be waxed, and *Tetrastichus planipennisi* bolts will not be waxed. Writing on the tops of bolts can be ignored; this helps Rearing Facility staff track exposure dates.

Oobius agrili will be sent as pupae in pill bottles referred to as Oobinators. Rarely, adult *O. agrili* will be shipped in plastic cups with honey streaked on the walls of the cup and sealed shut with plastic lids taped on and lined with filter paper.

Parasitoids should be released as soon as possible after receipt. When possible, all parasitoids should be released on the day they are received, or the next morning. After arrival, transport the parasitoids in the container to the release site. *Oobius agrili* do not fly as far as the larger larval parasitoids, so their release should be spread throughout the stand to enhance their establishment and dispersal.

If you are unable to release parasitoids as scheduled because of an emergency, follow the instructions below on how to care for them until you can conduct the release. However, if a significant delay occurs, or you find that parasitoids have died either in transit or by waiting, please contact the Biological Control Release Coordinator as soon as possible.

Care of Parasitoids if Release is Delayed

If there is an unforeseen delay caused by late delivery or unexpected weather conditions, place the cardboard containers in a temperature-controlled room. Do not leave the box near a window where they could be heated by the sun. Please unseal and open each box and determine what kind of parasitoid shipment you have received.

- **Ash bolts with immature larval parasitoids** can be held in closed bags and boxes.
- For **Oobinators** with immature *Oobius agrili* inside EAB eggs on paper strips, open the box and the plastic bag and keep them where they will not become overheated.
- ***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 Oobinators.
- **For cups with adult larval parasitoids (*Tetrastichus* and *Spathius*)**, each bag will contain several labeled cups containing small groups of live parasitoids. To maintain sufficient ambient moisture for the parasitoids, we recommend placing the rearing cups in a clear plastic storage tub with moistened paper towels. If the release delay will be over 24 hours, you can check each cup for the presence of honey. Honey provides the parasitoids with nourishment and some moisture during shipping. If no honey is visible on the lids of the cups, put 2-3 very small drops of honey on the lid and gently smear it. Be careful to avoid large drops of honey, which the parasitoids will drown in. If adding honey, open lids very carefully so adults do not escape.

Transporting Parasitoids to Field Sites

Carry the cups or infested logs inside the boxes when transporting parasitoids to the field for release. For delayed releases, they do not need to be re-bagged for local transport. **Care should be taken to keep the box out of direct sunlight or other potentially hot environments (e.g., a sealed vehicle). Keep the box in the shade –** parasitoids are extremely sensitive to overheating when confined. The trunk of a vehicle will suffice, but an

air-conditioned interior is even better, provided the vehicle will not be allowed to sit unattended in the sun for any period. 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 adult parasitoids carefully and avoid sudden movements. Adult parasitoids are susceptible to drowning in droplets of water or honey if the cup is inadvertently shaken or dropped.

FIELD RELEASE OF PARASITOIDS

A cup with adult *Spathius galinae*.

Ash bolt with parasitized EAB hanging from a tree.

An Oobinator hanging from a branch.

Adult Parasitoids in Plastic Cups

If possible, release the parasitoids in the morning or evening so they can move about in the environment before the onset of high afternoon temperatures. Gently tap the cup before opening. This causes the adult parasitoids to drop down to the bottom and avoids injuring any that are gathered near the lid as you open the cup. Carefully remove the snap-top lid with the filter paper liner. Place the cup and filter paper next to the trunk of an appropriate ash tree. On warm sunny days, most of the parasitoids will crawl up to the lip of the cup onto the tree trunk or simply fly away. On cooler days, most of the parasitoids

will remain in the cups. To dislodge these parasitoids, hold the cup upside down at a slight angle against the tree trunk and gently tap the cup against the tree, causing the parasitoids to jump or fly onto the tree trunk. You may find a small paintbrush useful for gently guiding any *Spathius* or *Tetrastichus* that refuse to leave the cup. Move the cups from tree to tree to ensure the parasitoids are somewhat evenly distributed throughout the release site. This is especially important for *Oobius agrili* as they do not fly as far as *Spathius* or *Tetrastichus*.

Larval Parasitoid Pupae in Small Ash Bolts

Bolts containing parasitoid pupae will need to be attached to the trunk of a suitable ash tree. Twine, zip ties, wire, and aluminum nails are commonly used to help hang the bolts on the trunk or branch. These materials are not provided by the Rearing Facility. Care should be taken to try to minimize direct contact between the bolt and the tree trunk because parasitoids can emerge from all sides of the bolt. Do not hang more than one bolt per tree if possible; the parasitoids will establish better if they are spread out. Choose trees that are alive, and trees with signs of active EAB infestation are preferable to trees with no signs of EAB infestation. The bolts should remain in the field for at least 8 weeks to ensure that all the parasitoids have emerged as adults. **If using nails:** Please only use aluminum nails or remove the nails from the trees when you recover the logs, because nails will harm sawmill equipment if the trees are harvested.

Oobius agrili Pupae inside EAB Eggs in Oobinators

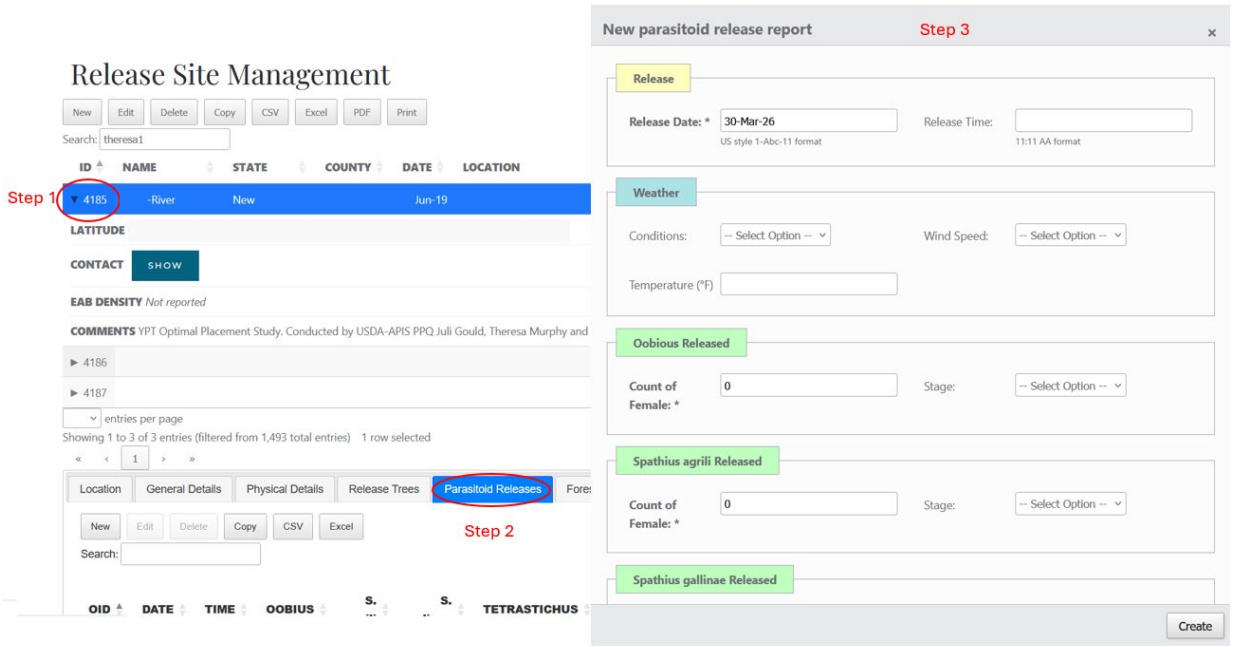
Twine, zip ties, wire, and aluminum nails are commonly used to help hang the Oobinators on the trunk or branch. These materials are not provided by the Rearing Facility. **If using nails:** Please only use aluminum nails or remove the nails from the trees when you recover the logs, because nails will harm sawmill equipment if the trees are harvested.

Hang one Oobinator per ash tree and distribute them widely throughout release sites to encourage the spread and dispersal of *O. agrili*. Choose trees that have evidence of fresh EAB attack such as fresh woodpecker feeding damage, live epicormic shoots along the trunk, or moderate canopy dieback (crown class 2-3; Appendix E). Ash trees with flaky or

coarse bark are preferred because they provide more oviposition sites for EAB to lay eggs; these are often the larger-diameter trees in the stand. Each Oobinator must be left on the trees for at least six weeks to allow all *O. agrili* adults to emerge. **Note: Oobinators will be shipped with caps on the vials. DO NOT forget to remove the caps before hanging the Oobinators in the field.**

ENTER RELEASE DATA INTO MAPBIOCONTROL

Every time you release parasitoids (i.e. for each release event), you will need to enter your release data. **Please enter release data either in real-time using the ArcGIS Field Maps app on your phone or tablet, or online at www.mapbiocontrol.org within 48 hours of release.** To enter data, search for and click on your site (Step 1) and then click on the 'Parasitoid Releases' tab (Step 2). A window will pop-up with a new parasitoid release report (Step 3 – see below for required fields).



Under the 'Release Tree' tab, there is also an option to add information about your specific release trees. This is optional, but you may record it on the website if you would like to keep track of where specific releases occurred within a site.

For each release event, record the following data (fields denoted with an asterisk are required by the website):

- **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)

Note: To estimate the number of parasitoids released, reference your parasitoid invoice. The tops of larval parasitoid bolts will also have a number that shows an estimate of females in that bolt. Oobinators and cups of adult parasitoids have the number of females written on the side or top.

***Oobius* Released**

- **Count of Female***
- **Stage Released** (Adult, pupae, both)

***Spathius agrili* Released** (if releasing as pupae in small ash bolts, this will be an estimate)

- **Count of Female***
- **Stage Released** (Adult, pupae both)

***Spathius galinae* Released** (if releasing as pupae in small ash bolts, this will be an estimate)

- **Count of Female***
- **Stage *Spathius galinae* Released** (Adult, pupae both)

***Tetrastichus* Released** (if releasing as pupae in small ash bolts, this will be an estimate)

- **Count of Female***
- **Stage *Tetrastichus* Released** (Adult, pupae, both)
- **Comments**

PARASITOID RECOVERY GUIDELINES

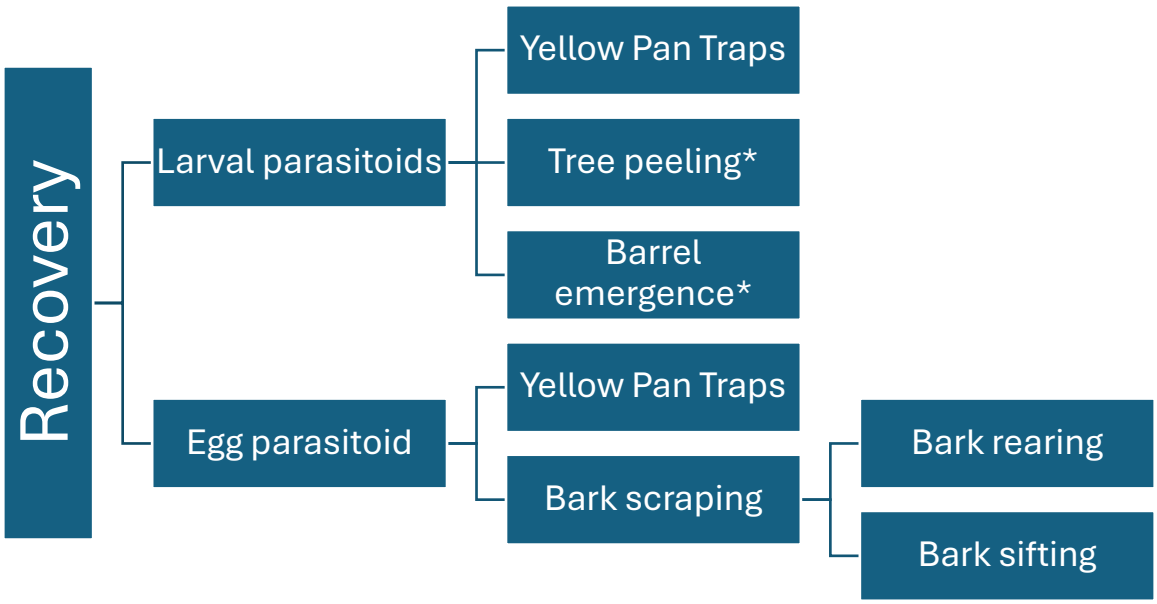
Program Site Release and Recovery Timeline
Years 1 and 2: Perform parasitoid releases.
Years 3 and 4: Perform parasitoid recovery efforts.

RECOVERY OVERVIEW

Recovery efforts should be started the year after releases are finished. You only need to sample one release site per county, although more is always better. **Two years of recovery work are standard** following your releases so that you can assess whether parasitoids are establishing. We define **establishment** as consistently recovering parasitoids at least two years after releases have finished.

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 certain methods but not others. Yellow pan traps are inexpensive and easy to sample, but they do not provide any measure of EAB parasitism. Collecting EAB eggs and larvae from trees allows us to calculate percent parasitism, but this method requires active sampling and may require felling trees for an adequate sample of EAB larvae. If resources permit, the best option is to use a variety of methods to ensure that if parasitoids are present, some will be recovered.

The graphic below illustrates which methods are used by the program to recover parasitoids. If you have released larval and egg parasitoids, please make sure you are making efforts to recover both. Yellow pan traps are the only method that can be used to sample for all parasitoids at once, but tree peeling and barrel emergence can be coupled with bark scraping so that you can recover both larval and egg parasitoids with those combined activities.



***You may scrape bark from trees you peel or logs you put in barrels for larval parasitoid recovery to also look for egg parasitoids.**

RECOVERY METHOD: YELLOW PAN TRAPS (YPTs)

Summary:

- Hang YPTs from ash trees showing current symptoms of EAB infestation.
- Deploy 15 YPTs per site.
- Deploy traps in the spring, summer, or early fall, depending on the parasitoid(s) you released, for at least 6 weeks.
- Change trap liquid every 5-7 days depending on the weather.
- Freeze samples that will not be processed right away, but do not refreeze thawed samples.

Many insects are attracted to the color yellow. Parasitoid recovery studies have shown that YPTs are effective at trapping all four of the introduced EAB parasitoids. Other known EAB larval parasitoids (e.g. *Atanycolus* spp.*,* native *Spathius* spp., *Phasgonophora sulcata*, and *Balcha indica*; see Appendix F) are also frequently trapped, along with many other species of insects.

Yellow Pan Trap Deployment Considerations

Choosing promising trees

Successful parasitoid recovery in YPTs seems to depend on where they are placed, and some trees are better for recovering parasitoids than other trees. Often, we find the same trees will catch parasitoids week after week, while nearby trees will recover nothing. We are not sure of all of the conditions that make an ideal tree, which is why we recommend placing multiple traps. **The trees you select must show some symptoms of current EAB infestation (e.g. fresh woodpecker feeding, epicormic shoots) with moderate to major signs of canopy dieback.** Do not put the traps on dead or healthy ash trees, since parasitoids will not be foraging on these trees.

YPTs seem to have the greatest success under the following conditions:

- On trees with visible signs of recent EAB damage (e.g. fresh woodpecker holes on the lower half of the tree) and live phloem.
- In sites with moderate to high canopy dieback (10-80%).
- In sites with ash along a forest edge (e.g. greenways, bike paths, roads).
- On trees that get a lot of sunlight, or on the sunny south/west facing sides of trees.

Due to the dynamics of our forested areas, it is not always possible to find "ideal" trees, and a given tree will only be suitable for hanging YPTs for a few years. Each year, do the best you can to find live but infested trees on which to hang the traps. If possible, select a variety of sizes of ash on which to place the traps. *Tetrastichus planipennisi* cannot parasitize EAB in ash larger than ~6 inches DBH, *Oobius agrili* are more likely to be found on trees with flaky bark (as in larger trees), and *Spathius* species can be found on trees of varying sizes. Distribute YPTs on ash trees throughout the release areas and, if possible, place some traps at or near the release trees.

How many YPTs should I deploy?

Deploy a total of 15 YPTs (one trap per tree) at your release site. Please know that trapping can generate a substantial sample load that will need to be processed. Samples can be frozen until able to be processed. If staff hours are limited, you may want to consider fewer sample dates or fewer sites. **WE DO NOT RECOMMEND PUTTING OUT FEWER THAN 15 YELLOW PAN TRAPS WITHIN A SITE.**

When should I deploy YPTs in the field?

Deploy YPTs at release sites in the year after your final parasitoid releases. For example, if you finish releases in August, you can start YPT recovery the following spring.

The timing of possible parasitoid captures in YPTs will depend on the climate in your area and the adult flight period of each species. Growing degree day base 50°F (GDD_{50F}) estimates are based on EAB phenology models and may not be accurate for geographic regions where we have less data available. See below for more specific guidance by species.

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

If you can do intensive sampling, we recommend deploying YPTs by 200 GDD_{50F} and sampling throughout the spring, summer, and early fall until 2900 GDD_{50F}. This requires visiting sites weekly, collecting the samples, and replacing the liquid in the bowl for the next week of sampling. A few studies have found the highest captures for larval parasitoids *T. planipennisi* and *S. galinae* occurred in the spring so if you can't do intensive sampling, then we recommend deploying YPTs by 200 GDD_{50F} and sampling for at least 6 weeks.

When to deploy YPTs for Spathius agrili

If you are trapping for *Spathius agrili* in the southern U.S., deploy the YPTs when 3rd-instar larvae become available. This could be as early as mid-June (in southern states such as Arkansas), or mid-July in more central states (such as Tennessee).

When to deploy YPTs for Oobius agrili

To capture *Oobius agrili* we recommend deploying the YPTs by 720 GDD_{50F} and trapping until 2200 GDD_{50F}. Petrice et al. (2021) found peak *Oobius agrili* captures at 1444-1598 GDD_{50F}. If you can't do intensive sampling, then we recommend having the traps out during this peak window.

How long should I leave YPTs in the field?

YPT samples should be collected and the liquid replaced every 5-7 days. 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 heavy rain, you might want to consider collecting the samples early. In limited cases, cooperators have found that if they increased concentrations of propylene glycol to 50%, samples could go up to 2 weeks before collecting. Care must be taken to test that this method will work at your site. If you find samples drying out or decaying, restart weekly collections. When servicing traps, collection bowls must be filled to the overfill hole to prevent them from drying out. In addition, if you get a heavy rain event, empty traps before or soon after because rain will both displace the solution from the bowl and dilute the remaining solution, which can lead to rapid decay of insect specimens.

Assembling and Deploying Yellow Pan Traps

Materials needed to build YPTs:

- Two 12-oz, 7–8-inch bright yellow plastic bowls
- One 8-inch (8 x 6 or 8 x 10) right-angled shelf-bracket
- Three 1.25-inch-long wood screws
- Two mini binder clips for securing the collection bowl to the holding bowl
- A one-hole punch and a utility knife for altering the bowls
- Fine-mesh screening (e.g. organza or no-see-um mesh)
- A hot glue gun
- Weather-proof marking pen (e.g. Sharpie) or grease pencil (needed if bowls are wet)
- Three 6-inch zip-ties (thin enough to fit through the holes on the shelf bracket)
- 20% solution of **clear** (not pink or green) **food grade** and/or **USP grade** propylene glycol (non-toxic antifreeze) diluted with water. It is easiest to buy pure propylene glycol and dilute it yourself
- Rechargeable portable electric screwdriver with bit and extra battery pack
- Unscented clear dish detergent

To request a kit for making YPTs or detailed instructions on how to construct them, please email Theresa.c.booth@usda.gov.

How do I set up the YPTs?

1. Using an electric screwdriver, attach the shelf bracket to the tree trunk approx. 5 feet above the ground, with 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 remains level.
2. Attach the bottom yellow "holding bowl" to the top side of the bracket with zip ties threaded through the three shelf bracket holes. Do not pull zip-ties too tightly to avoid distorting the holding bowl.
3. Set the top collection bowl in the holding bowl. Fill the collection bowl ~¾-full with 20% propylene glycol solution.
4. Add one drop of unscented dish detergent to the solution to break the surface tension, causing insects to become entrapped in the liquid.
5. 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.

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

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

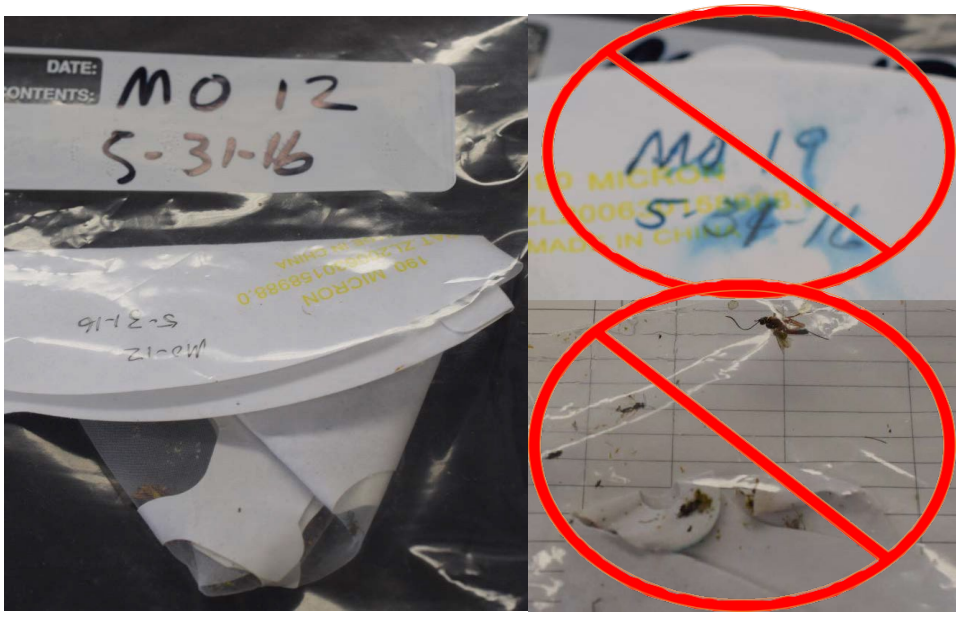
Servicing Yellow Pan Traps

Materials needed to collect YPT samples:

- 190-micron fine mesh paint filters
- Zipper top or whirl-pak plastic bags
- Pencils **(not pens)**
- 20% solution of **clear** (not pink or green) **food grade** and/or **USP grade** propylene glycol (non-toxic antifreeze) diluted with water. It is easiest to buy pure propylene glycol and dilute it yourself
- A squirt bottle with water for dislodging insects from the bowl or from leaves/slugs/debris
- A cooler to store samples in during transit

How do I collect insect samples from YPTs?

1. After locating the YPT in the field, label your paint filter with the site, date (including year), and YPT ID # in pencil (pen or marker may wash out).
2. 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.
3. Fold over the open end of the paint filter and place each one separately into a zippered bag. If the filter is labelled in pencil, you do not need to label the bag. If you cannot label the filter paper (because it is too wet, for example) then label the bag instead with Sharpie.
4. If it is hot, bring a cooler with ice packs while collecting samples 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.
5. Store samples in the refrigerator and process them within one or two days. If processing is delayed, store the samples in the freezer.



Label the filter paper in pencil (left) to avoid ink smearing (right). Fold the filter paper over (left) to avoid insects spilling out prior to processing (right).

Processing Yellow Pan Trap Samples

Processing YPT samples should be done locally, and suspect parasitoids you think may be EAB parasitoids must be sent for positive identification. To assist you in sorting the insects in the YPT samples, the Rearing Facility will send reference specimens of each EAB parasitoid species included in YPT kits. Until you are very comfortable identifying the parasitoids, it is a good idea to look through your reference specimens right before processing your pan traps to familiarize yourself with the look and size of the insects you are searching for. While you may be looking at your reference parasitoids under high magnification for detailed identification, also remember to look at your reference parasitoids under the lowest magnification that you will be using as you look through your samples to develop a 'search image'.

Materials needed to sort through samples:

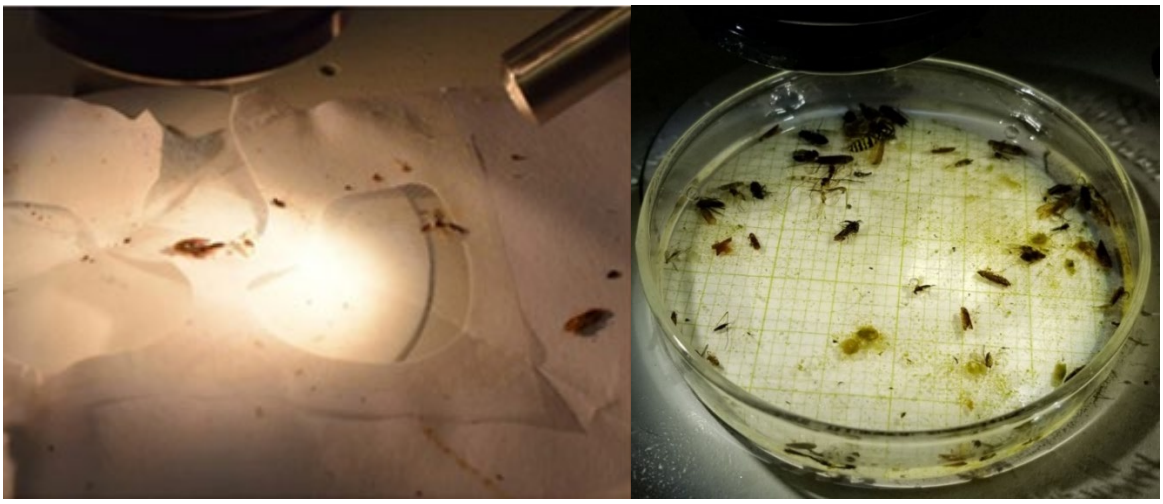
- Dissecting microscope
- Two pairs of fine forceps
- A fine 0, 00, or 000 paintbrush
- 95% ethanol (for preserving specimens)
- Small screw top vials (for specimens that need confirmation ID)
- A glass or plastic Petri dish to hold insect samples during the sorting process
- *Optional* 1% saltwater or 50% ethanol (for going through samples)
- *Optional* A small dropper to help add or remove specimens from the vials
- Datasheet (https://www.aphis.usda.gov/sites/default/files/ypt-recovery-datasheet.xlsx)

Step 1. Prepare your samples for processing.

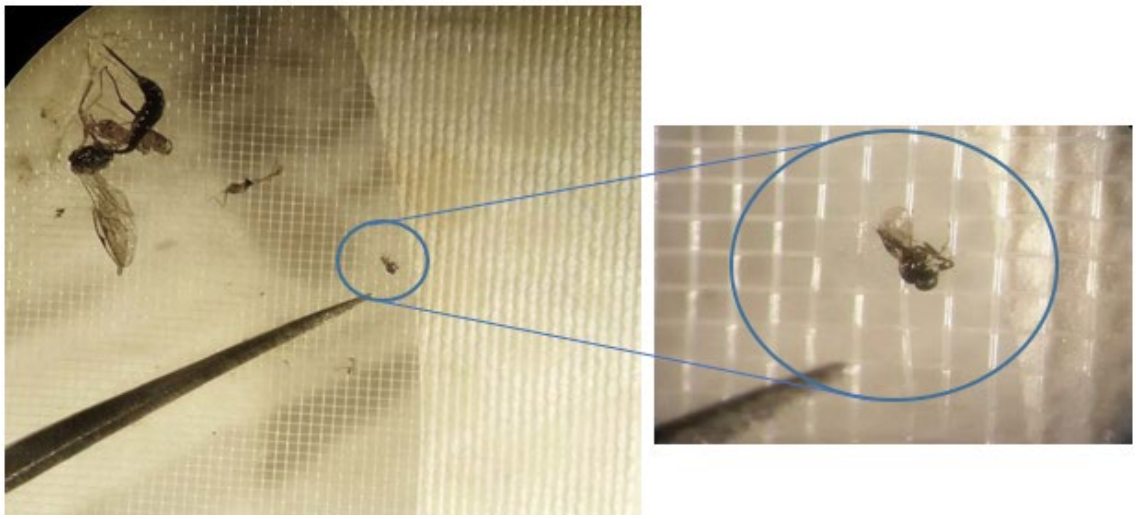
- If you stored your samples in the freezer, give them time to thaw before processing them. Try not to refreeze samples once thawed to help preserve the insects. If you have already thawed them, you can leave the samples in the refrigerator for a day or two while you process them.
- To start, open the zippered bag and record the location, date of sample collection (including year), and YPT ID number on your data sheet. Carefully unfold the filter paper, pull the filter apart at the seam, and dump the contents into a Petri dish. Check the bag and outside of the filter to make sure insects have not slipped out.

Step 2. Sort through insect specimens.

- Systematically sort through the insects caught on the filter under the microscope at the lowest magnification, looking for anything that looks like the EAB parasitoid species (see section below, "Overview of identifying EAB parasitoids in YPTs"). 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 "Overview of identifying EAB parasitoids in YPTs," Appendix C, and your reference specimens from the Rearing Facility.
- Once you finish checking the filter paper, check the Petri dish containing all the larger insects that you dumped out. Systematically go through the sample, sweeping top to bottom and then gradually left to right until you look through the entire sample. We find it helpful to touch each insect and move it aside after determining that it is not a suspect EAB parasitoid. It may be possible for smaller insects to stick to a larger insect or to some of the leaves or debris in your sample, so be sure to flip over insects and leaves and spread out debris to make sure you are not missing any small insects. Be sure to check the legs and tarsi of larger insects as parasitoids often become tangled and trapped there. This commonly occurs with *O. agrili* due to its tiny size.
- You may find it helpful to add a 1% salt solution or 50% ethanol solution to help break up the sample, so everything is not clumped together. Some processors find it helpful to add a grid to the bottom of the dish to help 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.



Left: A filter paper spread out and ready for viewing under the microscope. Right: A sample that has been poured onto a glass Petri dish ready for viewing under the microscope. This sample has alcohol added to help separate the insects and grid paper underneath the dish to help provide reference points for sorting through the sample.



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

Step 3. Preserve suspect EAB parasitoids.

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

1. MapBioControl Site ID#
2. Collection Date
3. Recovery ID # (This is generated in MapBioControl after you enter a possible recovery on the website. See Step 4 below)
4. Town and State

Step 4. Enter the data into MapBioControl.

(Refer to section *ENTER RECOVERY DATA INTO MAPBIOCONTROL*.)

- The Recovery ID# is generated when you create a recovery entry in MapBioControl. **BEFORE you send your samples, please create your recovery entries in MapBioControl and write the Recovery ID# on the paper in the vial with the insects**.
- Data for **ALL YPTs** at **ONE SITE** for the **SAME COLLECTION DATE** should be consolidated into **ONE** Recovery entry. Trap specific information can be included in the comments section if desired.
- Because you can have more than one vial on a given date at a site, these vials will have the same Recovery ID#. Include the MapBioControl Site ID# with each sample sent for cross reference.

Step 5. Ship your suspect EAB parasitoids for ID confirmation.

- Once you have several specimens for identification, ship them overnight to:

Andrea Anulewicz
USDA APHIS PPQ
5936 Ford Ct, Ste. 200
Brighton, MI 48116
Andrea.Anulewicz@usda.gov

- Ethanol in vials must be poured off prior to shipping due to shipping restrictions, but alcohol will be added back to the vials by the receiver.

Overview of Identifying EAB Parasitoids in YPT Samples

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

***Tetrastichus planipennisi* (Eulophidae)**

Tetrastichus planipennisi female. Photo by T. Booth USDA.

Morphological characteristics of *T. planipennisi* include:

- Dark bodied
- Red to dark red eyes
- Light-colored legs with dark femurs
- Smooth thorax with two lateral grooves on middle thoracic segment

Female *T. planipennisi* have the following diagnostic characters:

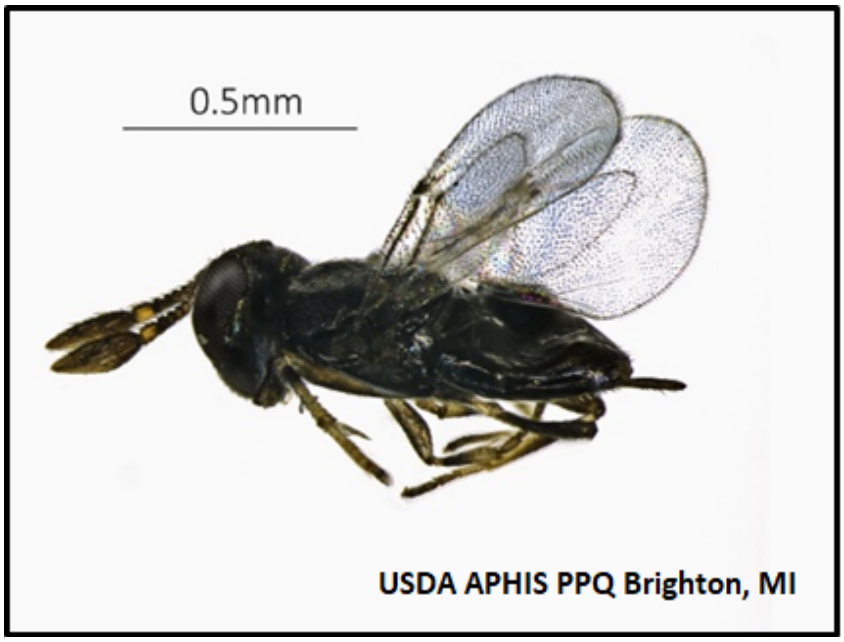
1. 6-segmented antennae
2. Abdomen longer than thorax
3. Last segment of abdomen is 3-4x as long as it is wide at the base

Male *T. planipennisi* have the following diagnostic characters:

1. 7-segmented antennae with clusters of longer hairs that look like ‘eye lashes’
2. Last segment of abdomen not elongated

***Oobius agrili* (Encyrtidae)**

Oobius agrili released for EAB biocontrol are all females. (This species is parthenogenic, i.e. they do not need to mate, and they only produce daughters.)



An adult female *Oobius agrili*.

Morphological characteristics of *O. agrili* include:

- Tiny size (~1 mm long)
- Compact, stout body with no waist
- Visible, short ovipositor
- Dark bodied with blue-green sheen

Oobius agrili can be identified by the following diagnostic characters:

1. Second-to-last antennal segment is light-colored; all other segments are dark
2. Clava (antennal “club”) is tapered upwards to a blunt point when the insect is viewed in profile

***Spathius agrili* and *Spathius galinae* (Braconidae)**

Left: *Spathius galinae*. Right: *Spathius agrili*. Photos by T. Booth USDA.

There are many native species of *Spathius* in the United States that can easily be confused for the two released species. The following morphological characteristics will help you distinguish between our introduced parasitoids and native species.

Morphological characteristics of S. *agrili* and *S. galinae* include:

- Reddish brown bodies with dark brown or black abdomen
- Spherical head with spherical eyes
- Back of head smooth, without wrinkles
- Wings with brown veins
- Very long antennae with >26 segments
- Short pedicel (narrow waist connecting thorax and abdomen)
- Long ovipositor (in females)

Spathius galinae can be identified by the following diagnostic characters:

1. Brown-tinged forewings with obvious clear spots
2. Base of all tibia yellowish; 3/5ths base of hind tibia yellow

Spathius agrili looks very similar to *S. galinae*, except the forewings have clear bands rather than clear spots, and the legs and body are generally darker in color.

RECOVERY METHOD: TREE PEELING AND DEBARKING

OVERVIEW:

A. **Sampling for larval parasitoids (*T. planipennisi* and *Spathius* spp.):**
   - Felling trees and peeling logs
   - Peeling standing trees

B. **Sampling for egg parasitoid (*O. agrili*):**
   - Bark scraping cut logs
   - Bark scraping live trees
     - Sheet method
     - Tote method
   - Processing bark samples
     - Rearing bark samples in tubes
     - Sifting bark samples

Peeling and debarking 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. We recommend felling trees for peeling and/or debarking between November and March.

Sampling for Larval Parasitoids (*T. planipennisi* and *Spathius* spp.)

Felling and Peeling Logs to Recover Larval Parasitoids

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

If felling trees, select at least four trees within or near where parasitoid releases occurred that are alive (based on bark peeling and confirmation of live phloem), show signs of fresh damage due to EAB (woodpecker holes, bark splits, epicormic shoots), and are less than 10-inches DBH. At least two trees should be "small" trees under 4-inches DBH to capture *T. plannipennisi*. Give each tree a unique ID number and record its DBH, location (GPS coordinates), and the date the tree was felled. We generally cut these trees into 1-meter logs to make them easier to handle and peel.

Below is a guide to peeling ash logs to look for EAB. If you have questions about tree peeling or want some additional visual resources, please contact Theresa.C.Booth@usda.gov .

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

Materials needed for tree peeling:

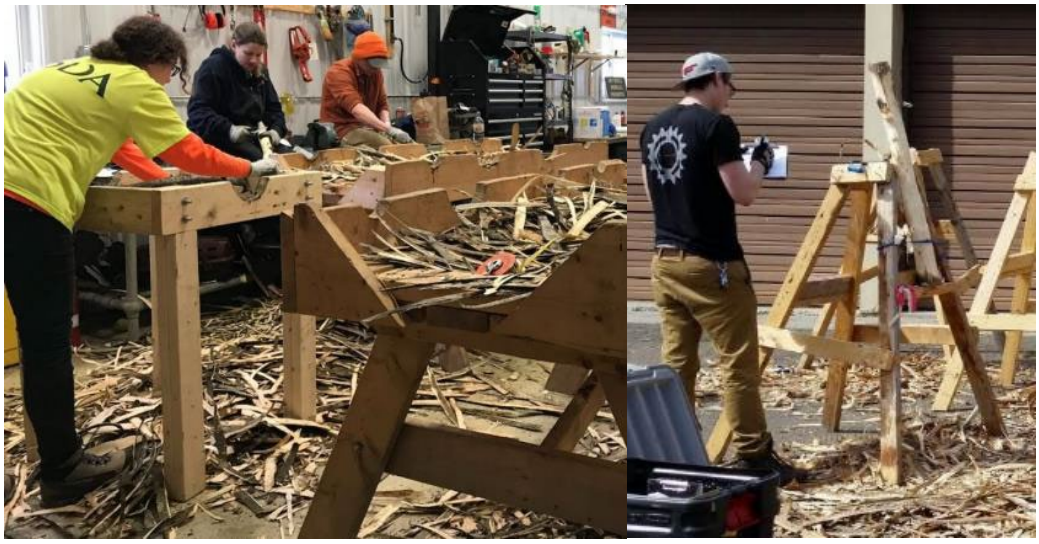
- Safety glasses
- Cut-proof gloves
- 9” drawknives
- 5” small drawknives
- Small knives and/or chisels
- Hammer to be used with a chisel to help dig into the wood for J-larvae (optional)
- Face shield or bandana for dust (optional)
- Peeling stand/table (good for large logs)
- Shave mule/jawhorse/C-clamps (good for smaller logs or branches)
- *Optional* Datasheet (https://www.aphis.usda.gov/sites/default/files/tree-peeling-datasheet.xlsx)

Step 1. Peel the ash logs.

- Logs are easiest to peel if debarked soon after felling.
- If you need to store the logs, try to store them in a way that reduces moisture loss. You can keep them in a cold chamber and/or store them in a barrel of water or seal the ends (ex. with Anchorseal®) to reduce moisture loss.
- It is easiest to peel using a large 9-15” drawknife.
- If the bark is too thick, you can remove the outer bark with a drawknife first. If you find an EAB gallery, carefully remove the phloem in that specific area. For more

precision, use a sharp chisel, small knife, or small 5” drawknife.

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



(Left) A peeling table. (Right) A peeling stand.

Step 2. Inspect all EAB galleries.

Examine the end of each gallery for EAB larvae or signs of parasitism. (See Appendix B for photos.) You may see the following fates:

- **EAB larva parasitized by *Tetrastichus*, which 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).
  4. have emerged from the EAB gallery, leaving only the head and tail of the EAB larva and small black spots in the gallery called meconium (the spots are waste excreted by *T. planipennisi* larvae before pupation is complete). The EAB larva head and tail may not always be visible.

  **Tetrastichus planipennisi* larvae remain inside the EAB larva for at least a week before emerging. If you have access to a laboratory, you may find more parasitized larvae if you place EAB late-instar and J-larvae in a petri dish and dissect them. If parasitized by *T. planipennisi*, many small eggs and/or larvae can be seen within in

the EAB hemolymph. Note: EAB larvae can be saved in labeled petri dishes, stored in the fridge, and dissected weeks later.

- **EAB larva parasitized by *Spathius*.** All *Spathius* life stages live on the outside of the host. *Spathius* eggs and small larvae are difficult to see with the naked eye. By late fall, most will be large larvae or will have spun multiple silken cocoons that are fairly easy to see within the EAB galleries.
- **EAB larva parasitized by a single solitary larva or cocoon.** An EAB parasitized by a single larva or cocoon is probably *Atanycolus* (native parasitoid) or *Balcha indica* (naturalized parasitoid) (Appendix F). You do not need to ship these parasitoids.
- **A healthy EAB larva.** If you find a healthy EAB larva that is a small instar (1st or 2nd) it does not need to be dissected. If you find a healthy EAB larva that is a larger instar (3rd, 4th, or J-larva) you can dissect it for signs of internal parasitism. A J-larva is not going to be visible until you chisel into the wood of the tree.
- **An empty gallery where a woodpecker has taken the larva.** Use a Sharpie to mark woodpecker holes before peeling so you know why the gallery you've peeled is empty. Recording data on woodpeckered EAB is not required.
- **A dead EAB larva.** EAB can die for a variety of reasons including tree defenses, fungi, viruses, or extreme cold.

Step 3. Extract and ship suspected parasitoids.

- If you find a potentially parasitized EAB or parasitoid clutch, carefully remove the EAB larva and any parasitoid eggs, larvae, and pupae/cocoons, and place them in a small petri dish with a friction-fitting lid (Fisher Scientific 50 x 9-mm dishes – catalog no. 08-757-105 is a good choice) or a vial with a capacity of at least 1 dram. Please do not use vials with very tiny openings since identifiers will need to insert forceps to remove the insects.
- Save each clutch of parasitoids and/or each parasitized EAB larva in a separate vial or petri dish.
- Once you enter the data into MapBioControl, it will generate a Recovery ID #. **Please include that Recovery ID # on shipments you send.** This number will be used by the identifier to enter the parasitoid ID into MapBioControl.
- **Using a pencil**, label a small piece of paper with the state, Recovery ID #, Site ID #, tree number, and date (Use Day-Month-Year in format: DD-MMM-YYYY; ex.10-Jul-2023) and place the paper into the vial or petri dish. Pencil is preferred as pen and Sharpie can be washed away by ethanol.

- Ship the specimens for identification within 48 hours to:

  Andrea Anulewicz
  USDA APHIS PPQ
  5936 Ford Ct, Ste. 200
  Brighton, MI 48116
  Andrea.Anulewicz@usda.gov

  If you need to keep samples longer before shipment, add 95% ethanol to preserve the specimens and then pour off the ethanol before shipment.
- You may also wish to take photos of each parasitoid clutch and/or any suspect signs of *T. planipennisi* meconium and email those photos to Andrea Anulewicz.

Step 4. Enter the data into MapBioControl.

(Refer to section *ENTER RECOVERY DATA INTO MAPBIOCONTROL*.)

- Once you enter the data into MapBioControl, it will generate a Recovery ID #. **Please include that Recovery ID # on shipments you send.**
- Peeling 4 trees at one release site will count as **one** recovery entry.
- Record your peeling event and if you have found potential parasitoids. **Please make a record on MapBioControl even if you do not find parasitoids**. Negative data is just as important as positives. If you found parasitoids, please note in the comments how many potentially parasitized clutches you are shipping.

***Note: Count the number of EAB larvae that were parasitized, not the number of parasitoids in each gallery. Each group of parasitoids in a single EAB gallery counts as ONE clutch. Please ship each clutch in a separate vial or petri dish.**

Peeling Standing Trees for Larval Parasitoids

This is a modified version of the previous section for if you want to peel trees without felling them. You will need the same materials and follow the same methods as above, but peel only on the sections you can reach. If peeling standing trees, debark the lower 2 m of the trees. **Sample at least four trees** but consider sampling more since you are only peeling the lower part of the tree, especially if your initial four trees do not yield parasitoids.

Sampling for Egg Parasitoid (*Oobius agrili*)

Choosing Promising Trees

Bark sampling can recover EAB eggs that may be parasitized by *O. agrili*. It is not worth sampling for EAB eggs if the log shows no signs of fresh EAB infestation. If you are unsure about a tree or log, you can peel a small section to see how many galleries are underneath the bark.

- You are more likely to find EAB eggs by sampling a part of the tree trunk that shows signs of recent EAB infestation and is still alive (i.e. the bark is still attached to the sapwood). Fresh woodpecker holes or blonding and/or live epicormic shoots are a great indicator of recent EAB infestation. If scraping a standing tree, it is recommended that trees have at least 5-10 fresh woodpecker holes within the first 3-4 meters above the ground (but the more the better).
- Choose an area with furrowed or flaky bark (smooth bark will not have many eggs).

To help reduce the burden of sifting bark samples, some collaborators have noted that they avoid peeling bark areas covered in moss/lichen because this adds a ton of debris that makes sorting those samples take significantly longer.

Bark Scraping Cut Logs

Bark samples can be taken from ash trees felled for other recovery work, but we recommend sampling at least 10 trees per site. So, if you are already felling four trees for larval parasitoid recovery, we recommend taking bark samples from additional trees that you do not fell, to reach 10 total. We suggest taking one to two bark samples per tree from a section of the tree that is likely to have EAB eggs (see above section, “Choosing Promising Trees”).

From each log section:

1. Mark off a vertical area of bark 10 x 100 cm.
2. Lay the log down and use a draw knife to carefully shave the outer layer of bark into the tray. A non-plastic tray is best to avoid static that can affect bark and EAB eggs, but a plastic tray can be used if treated with anti-static spray or a dryer sheet.
3. Pour each bark sample into a large paper lunch bag (Masking tape the bottom of the lunch bag to prevent bark/eggs from spilling out).
4. Label the bag with the date, site, state, log number, and DBH.

5. Rear and/or sift the bark samples as described below.

Bark scraping a cut log for *Oobius agrili*. Photo by W. Davidson, NH Department of Forest and Lands.

Bark Scraping Live Trees

You can also take bark samples from living, EAB-infested ash trees in the vicinity of the original release site in the field (see above section, “Choosing promising trees”).

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, use one of the following methods:

Sheet method (requires two people):

1. Wrap a piece of heavy plastic sheeting around the base of the tree and secure it with duct tape. Have one person hold the edges of the sheeting up in the shape of an inverted cone.
2. Using a drawknife, the other person shears off a thin layer of outer bark within the delineated area, and the bark debris will fall into the inverted plastic cone.
3. Remove the duct tape and, using the plastic sheeting, funnel the bark sample into a paper lunch bag (reinforce the bottom of the lunch bag with masking tape to prevent bark/eggs from spilling out).
4. Label the sample with the date, site, state, tree number, and DBH.

Tote method:

1. Modify a 12-to-18-gallon plastic tote:
   a. Cut the lip on one side of the tote and put duct tape over the cut edge. This will allow the tote to sit flush against the tree with no gap.
   b. Add extra duct tape around the corners to reinforce them. The corners are the points most likely to weaken and break from pushing against the tree.
   c. Cut two small holes on either side of the tote. Attach a rope to hold the tote around your neck while scraping.
2. In the field, press the tote into a suitable tree so that it sits flush against tree.
3. Using a drawknife, shear off a thin layer of outer bark within the delineated area. Slice off multiple shallow layers of bark until the entire area has been debarked.
4. Once a sample has been collected, dump it into a paper bag (reinforce the bottom of the lunch bag with masking tape to prevent bark/eggs from spilling out). Brush any residual bark from the tote into the paper bag.
5. Label the sample with the date, site, state, tree number, and DBH.

Bark sampling using the sheet method.

Bark sampling using the tote method.

Optional: Toby Petrice has designed a drawknife shield (green item in picture above) to help with bark scraping. If you're interested in this design, you can contact Toby Petrice for more details at toby.petrice@usda.gov.

Processing Bark Samples

Once you collect your bark samples, you have two options for finding *Oobius agrili* eggs:

- Keep samples in tubes to rear out any overwintering *O. agrili* adults that have not yet emerged.
- Sift samples to look for all EAB eggs, including any that were parasitized by *O. agrili*.

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 you are only rearing bark samples and not sifting, we recommend taking 30 bark samples per site.** If you are sifting, we recommend taking 10 bark samples per site.

Rearing Bark Samples

Oobius agrili overwinters inside EAB eggs as mature larvae and emerges in the spring. Bark samples can be reared in containers (i.e. rearing tubes or barrels) to collect adult *O. agrili* that may be overwintering in EAB eggs. These rearing containers can be constructed from cardboard poster tubes with tight-fitting plastic plugs.

Materials needed for bark rearing tubes:

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

Construction and collection from bark rearing tubes

1. The outer surface of plugs should be spray painted black. This will reduce light transmitted and direct emerging adult parasitoids to the opening.
2. Cut a 1.5"-diameter hole in the center of one of the plugs.
3. Cut a 1.5"-diameter hole in the cap of a 4.5 oz. specimen cup.
4. Glue the larger end of the funnel to the bottom side of the specimen cup lid (side with threads) using hot glue.
5. 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 there are no tiny gaps

between the plug surface and cap surface that would allow these small parasitoids to escape.

6. Screw the specimen cup onto the cap.
7. 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.
8. Hold bark in rearing containers for at least 6 weeks at room temperature to allow all parasitoids to emerge.

Sifting Bark Samples

1. Dry the sample for at least one month at room temperature. (If the bark was already processed for rearing *O. agrili* it will already be dry.)
2. Place small portions 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 pan. (Ceramic is recommended because it eliminates static electricity that makes working with eggs difficult.)
3. Using a dissecting microscope to sort through each bark sample, we recommend following the method described by the Minnesota Department of Agriculture. Their presentation, which includes many photos of parasitized EAB eggs and look-alikes, can be found at: Biological Control of Emerald Ash Borer: Bark Sifting for Oobius agrili.

(Left) A No. 14 covered soil sieve for sorting bark. (Right) A white ceramic baking pan with sifted bark ready for viewing under a dissecting microscope.

If you find EAB eggs or egg parasitoids:

- Collect all EAB eggs into a small Petri dish with a friction-fitted lid (Fisher Scientific 50 X 9-mm dishes – catalog no. 08-757-105 is a good option).
- Collect adult parasitoids into a vial with 95% ethanol (ethanol needs to be poured off before shipping).
- Label petri dishes with state, Recovery ID #, Site ID #, and date of collection (DD-MMM-YYYY, ex. 05-Feb-2024) using a fine-tip permanent marker. For ethanol vials, include information on a small slip of paper inside the vial (written in pencil to avoid smearing).
- Ship the EAB eggs and parasitoids collected from bark sifting or reared from bark or logs to:

Toby Petrice
US Forest Service
2601 Coolidge Rd, Ste. 203
East Lansing, MI 48823

RECOVERY METHOD: BARREL EMERGING FELLED LOGS OR BRANCHES*

Peeling logs or trees is a great way to directly measure EAB density and parasitoid presence/absence but takes a significant amount of time to complete. For a passive recovery method that is less time-intensive, you can emerge parasitoids from cut logs or branches in barrels. This method can theoretically be used for all parasitoids, but has had the best success with larval parasitoids. Consider bark scraping after barrel emergence to look for *Oobius agrili* if you do not find any during the emergence period.

**This method is still in development. If you've had success with barrel emergence, please consider sending feedback to Theresa.c.booth@usda.gov for incorporation into these recovery guidelines*.

Choosing Promising Trees

- Trees must be **alive** but infested with EAB. The higher the larval density, the better. Try to select trees around **4-6 inches DBH.**
- Smaller trees are more likely to host *Tetrastichus planipennisi* and are easier to fell, transport, and fit into barrels.
- Look for signs of infestation (fresh woodpecker feeding holes in winter or crown dieback, epicormic shoots, and fresh woodpecker feeding holes in spring/summer/fall).
- Make sure the tree is alive. You can **use a drawknife to peel back bark** to confirm the tree is alive and/or to assess infestation levels.
- You only need to take sections of the tree that show fresh signs of EAB infestation. If infestation is near the crown but the base is clean, skip collecting the lower logs. If the top is dead but the lower trunk is heavily infested, leave the dead portion and harvest only the live infested material.

Note: You may have to scout multiple parts of your site to locate suitable trees. The extra effort to find suitable trees yields better parasitoid recovery.

How to Build Barrels

- You can use any large barrel (25 to 55 gallons). Larger barrels can store more logs.
- Attached a small, transparent container with a lid (like a Mason jar) to the barrel lid to collect parasitoids.

- To attach the collection container, cut a hole through the container lid so that the parasitoids can enter the container. Then secure the container lid to the barrel so the container can be removed to collect the parasitoids. See above section, “Rearing bark samples for *Oobius agrili*” for some suggested materials and methods.

Examples of how collection containers have been attached to barrel lids to collect emerging parasitoids.

Barrels being stored in a rack.

Storage Conditions

- Leave the logs laid out for a few weeks to allow them to dry and prevent mold proliferation in the barrel. Make sure logs are labelled with the date, site information, and tree information for tracking parasitoid emergence.
- Place logs into barrels, giving enough space that parasitoids can still emerge between logs. Avoid tightly packing the barrel.
- Barrels can be stored and stacked horizontally to take up less space. You can buy a commercial barrel or drum rack or build one.
- Ideal ambient temperatures for emergence are 75-80˚F with 65-75% humidity. Room temperatures between 70-72˚F will allow parasitoid emergence, but at a slower rate and reduced number.

Labeling and Monitoring

- Label barrels with the date, site information, and tree information for tracking parasitoid emergence.
- Check barrels at least once per week for parasitoid emergence.
- Monitor emergence for at least 6 weeks at room temperature or through the next field season (until the end of August) if kept at outdoor temperatures.
- Collect the contents of the barrels after you remove the logs to ensure you get everything that didn't make its way into the collection jar. Shake the barrel out onto a large metal tray, then transfer loose material to a jar for sorting through.
- Optional: Logs can be peeled 3-4 weeks after attempting emergence to look for parasitoids stuck under the bark.

Collecting Parasitoids

- Collect parasitoids and ship at least a few adults from each emergence to the Rearing Facility for ID. The rest can be re-released if desired.
- Label the adults with state, Recovery ID #, Site ID #, and date of collection (DD-MMM-YYYY, ex. 05-Feb-2024). The collection date should be the date the trees were recovered from the field.
- Specimens should be sent to:

Andrea Anulewicz
USDA APHIS PPQ
5936 Ford Ct, Ste. 200
Brighton, MI 48116
Andrea.Anulewicz@usda.gov

ENTER RECOVERY DATA INTO MAPBIOCONTROL

Enter all recovery data, INCLUDING ALL NEGATIVE DATA ('zero' entries where EAB parasitoids were not recovered). 'Zero' data is extremely important because we need to know if parasitoids are not establishing at release sites.

Once you have completed surveys to detect established parasitoids, enter the data on the MapBioControl website or using the mobile app **before shipping samples for identification.** Once you make a recovery entry, MapBioControl will give that entry a Recovery ID #. It is the first number on the Parasitoid Recovery page, under "ID." **Please include this Recovery ID # in any samples you ship so that we can match it up to the appropriate record.**

To enter data on the website, go to www.mapbiocontrol.org and click on RECOVERY in the blue banner at the top. Click the 'New' button to enter new data. You will be asked to enter the following data **(* indicates required information):**

Identification Information

- **Site ID:** Enter the MapBiControl Site ID for your site. If you find parasitoids not connected with a particular site, leave this blank.
- **Trap ID:** If you wish to record which trap collected the insects for your own information, you can enter the trap number here.

Location Information

- **Country***
- **State(USA)/Province(Canada):***
- **Latitude ***(dd.ddddd): You may enter the LAT/LONG from your Site ID if you don't have specific recovery LAT/LONG.
- ***Longitude** (dd.dddd): You may enter the LAT/LONG of your Site ID if you don't have specific recovery LAT/LONG.

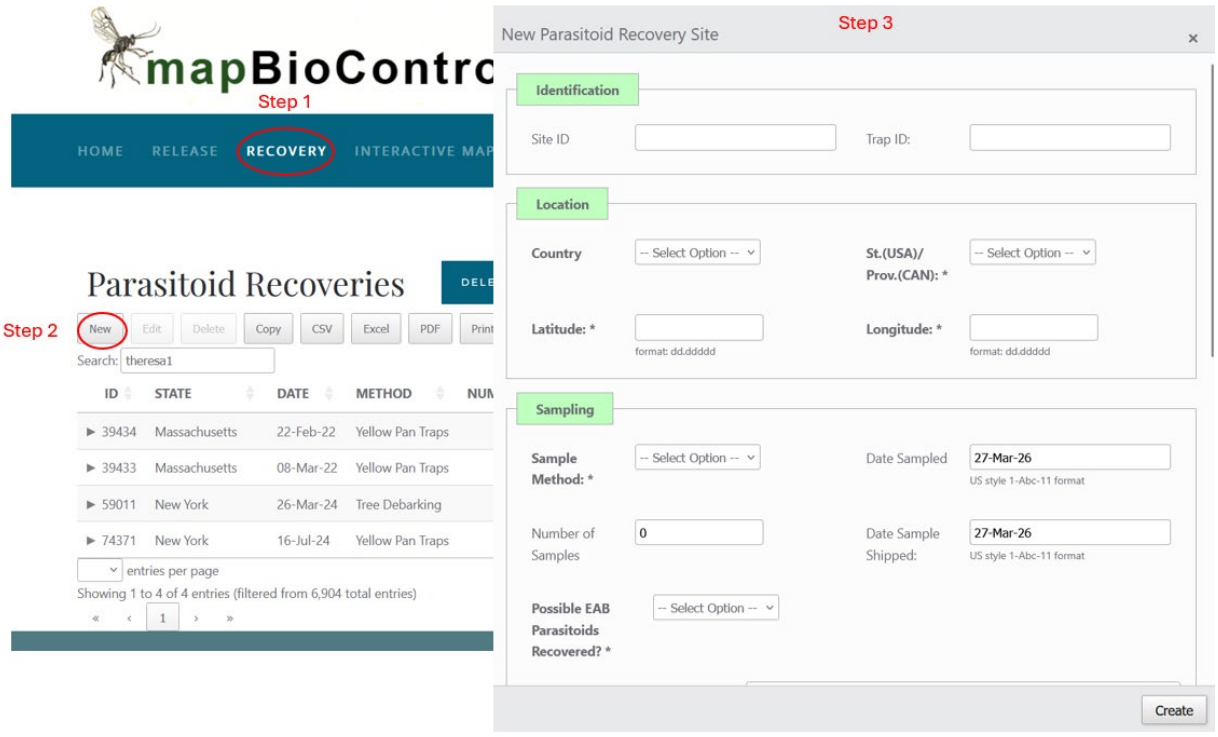
Sampling Information

- **Date sampled:** In format DD-MMM-YY, Ex. 20-JUN-23
- **Sampling Method*:** (Bark in Tubes, Bark Sifting, Burlap Trap, Egg Collection, Logs in Tubes, Sentinel Eggs, Sentinel Larvae, Tree Debarking, Yellow Pan Traps, Other)
- **Number of Samples:** If 'Yellow Pan Traps', enter the number of YPTs you sampled that week at that site (usually 15). If 'Tree Debarking', 'Logs in Tubes', 'Bark in

Tubes', 'Egg Collection', or 'Bark Sifting' enter the number of trees you sampled.

- **Date Sample Shipped**: In format DD-MMM-YY, Ex. 05-FEB-24. Leave blank if no parasitoids were recovered.
- **Samples Shipped to**: Who you shipped your suspect parasitoid samples to. This will most commonly be **Andrea Anulewicz**. Leave blank if no parasitoids were recovered.
- **Possible EAB Parasitoids Recovered?* '**Yes' if shipping suspected samples. 'No' if no suspect parasitoids were found/shipped. If needed, this field will be updated by the identifier after processing samples.
- **Comments:** It is very helpful if you write which parasitoids you thought you recovered in this field. Ex. "Suspect 2 *T. planipennisi* and 3 *O. agrili*". This will help the identifier determine what they are looking for in the vial.

NOTE: The actual number of parasitoids recovered can only be entered into the database by program identifiers, but you can write what you suspect in the comments.



PUBLICATIONS ON RECOVERY METHODS

Petrice, T.R., Bauer, L.S., Miller, D.L., Stanovick, J.S., Poland, T.M. and Ravlin, F.W., 2021. Monitoring field establishment of the emerald ash borer biocontrol agent *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae): Sampling methods, sample size, and phenology. *Biological Control*, *156*, p.104535. https://doi.org/10.1016/j.biocontrol.2021.104535

Petrice, T.R., Poland, T.M., Bauer, L.S., Strazanac, J.S., Duan, J.J., Schmude, J.M. and Ravlin, F.W., 2023. Factors affecting yellow pan trap captures of the emerald ash borer biocontrol agents *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae): implications for monitoring establishment and seasonal abundance. *Biological Control*, *184*, p.105272. https://doi.org/10.1016/j.biocontrol.2023.105272.

Rutledge, C.E., Van Driesche, R.G. and Duan, J.J., 2021. Comparative efficacy of three techniques for monitoring the establishment and spread of larval parasitoids recently introduced for biological control of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). *Biological Control*, *161*, p.104704. https://doi.org/10.1016/j.biocontrol.2021.104704

Parisio, M. S., Gould, J. R., Vandenberg, J. D., Bauer, L. S., & Fierke, M. K. (2017). Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biological Control, 106, 45-53. https://doi.org/10.1016/j.biocontrol.2016.12.009

Jennings, D. E., Duan, J. J., & Shrewsbury, P. M. (2018). Comparing Methods for Monitoring Establishment of the Emerald Ash Borer (*Agrilus planipennis*, Coleoptera: Buprestidae) Egg Parasitoid *Oobius agrili* (Hymenoptera: Encyrtidae) in Maryland, USA. Forests, 9(10). https://doi.org/10.3390/f9100659

Abell, K. J., Bauer, L. S., Duan, J. J., & Van Driesche, R. (2014). Long-term monitoring of the introduced emerald ash borer (Coleoptera: Buprestidae) egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae), in Michigan, USA and evaluation of a newly developed monitoring technique. Biological Control, 79, 36-42. https://doi.org/10.1016/j.biocontrol.2014.08.002

EAB life stages

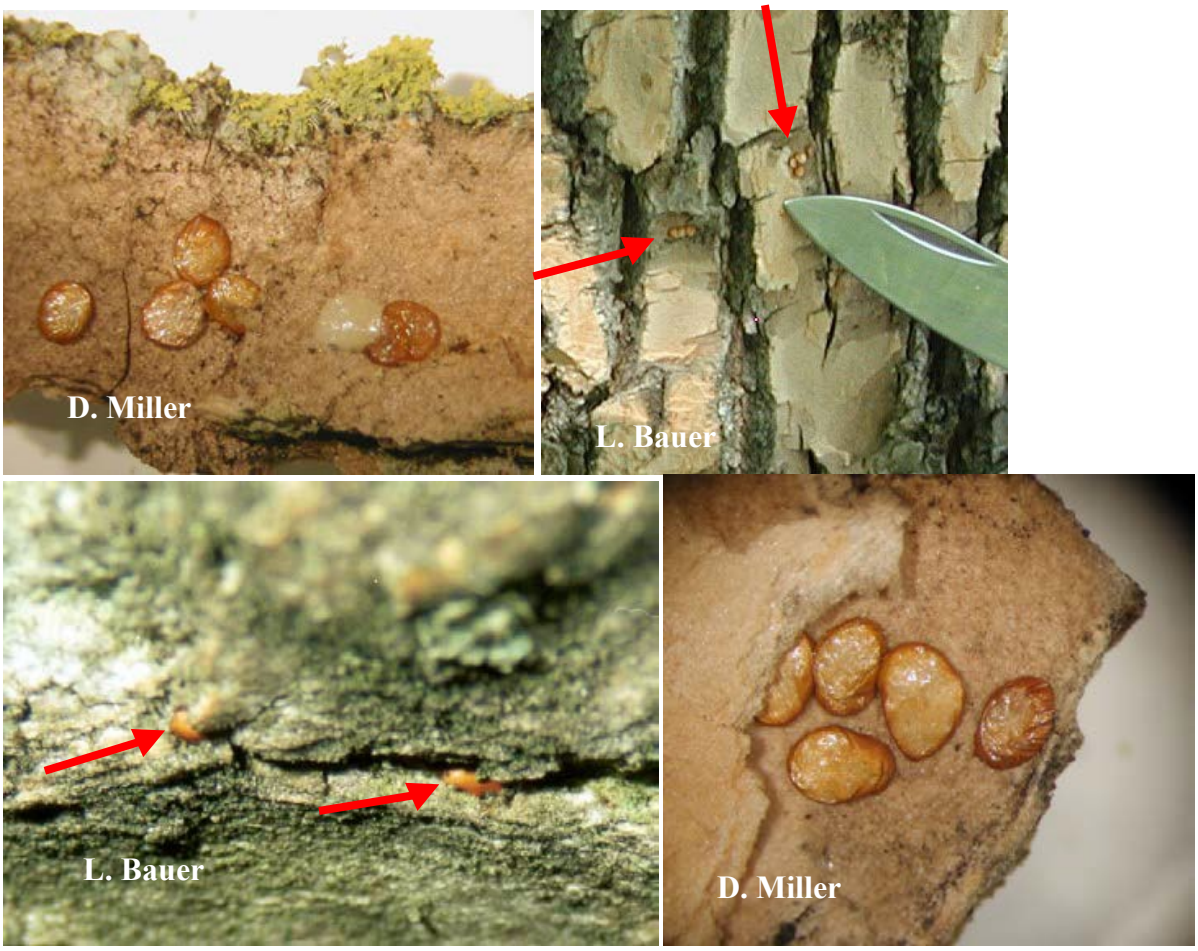


Figure A1. EAB eggs. (Top left) The white egg is newly laid, while the other eggs have matured to an amber color. (Top right) Clusters of eggs laid between bark flakes that have been exposed with a knife. Arrows indicate the eggs. (Bottom left) Single eggs laid in bark crevices. Arrows indicate the eggs.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

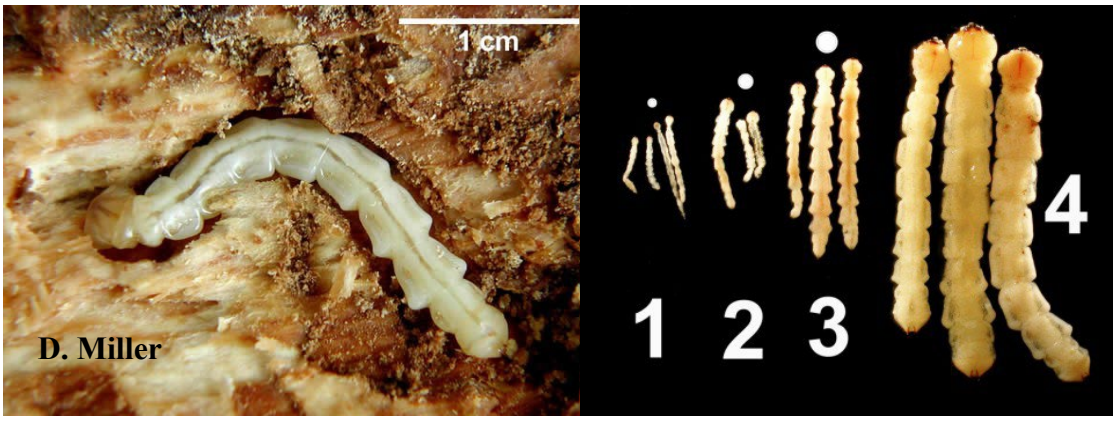


Figure A2. EAB larvae. (Left) Mature 4th instar EAB in a gallery. (Right) The four instars of EAB larvae.

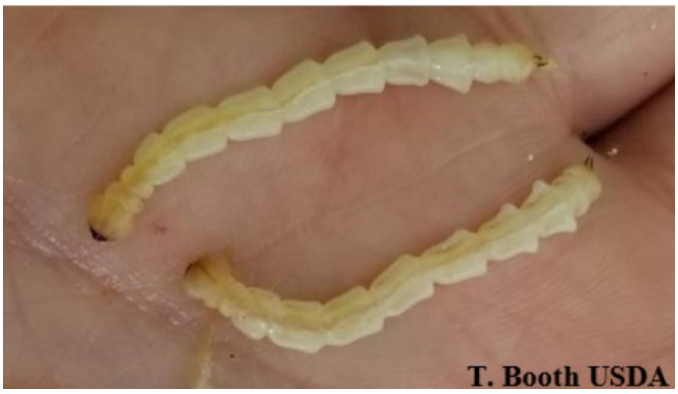


Figure A3. EAB larvae. Two 4th instar EAB larvae. Larvae are dorsoventrally flattened and have 10 bell-shaped segments and a posterior pair of small brown structures called urogomphi.

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

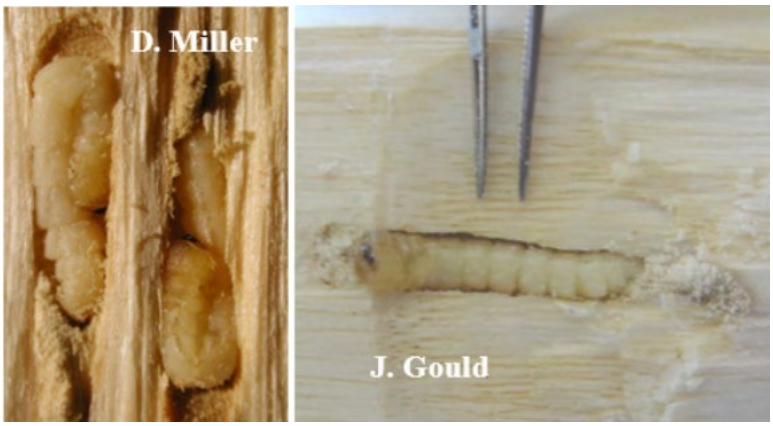


Figure A5. Overwintering EAB larvae. (Left) J-larvae in pupal chambers in outer sapwood. (Right) Pre-pupa in outer sapwood.

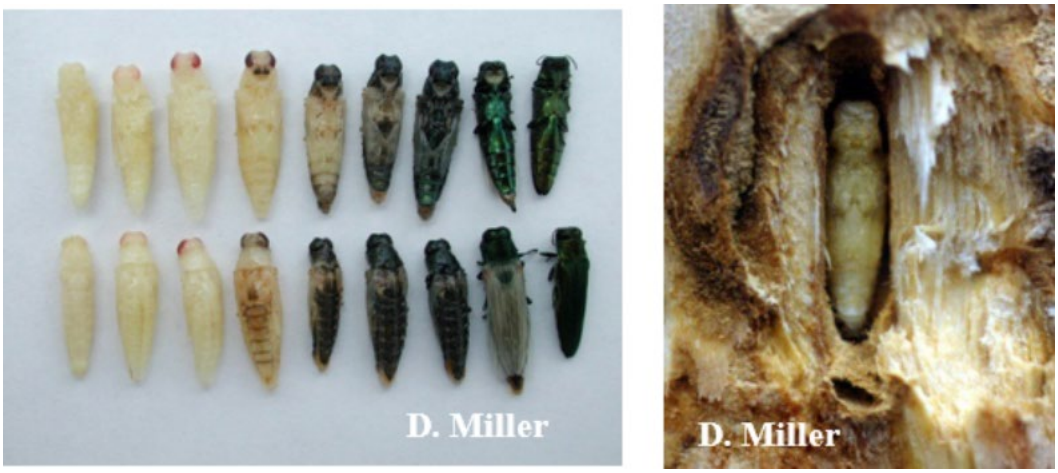


Figure A6. EAB pupae. (Left) Stages of maturing pupae from left to right. (Right) Pupa in outer sapwood.

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

Figure A8. External signs of EAB overwintering chambers under the bark. (Left) An EAB gallery filled with light-colored frass, indicating an overwintering EAB larva is underneath in the sapwood. (Right) The outer sapwood has been peeled away to show the exit portals of three pupal chambers, each with 2 holes filled with frass. Arrows indicate the portals.

Signs of EAB infestation

Figure A9. Thinning ash crowns. Two photos showing thinning ash crowns due to stress from EAB infestation.

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

Figure A11. Bark splits with EAB larval galleries beneath the bark. (Left) Bark split with larval gallery visible beneath the bark. (Right) Exposed bark split after bark is peeled off tree. Note callusing around the old gallery.

Figure A12. Woodpecker feeding on EAB larvae. (Left) Hole from where a woodpecker fed on an EAB larva. (Center) "Blonding" caused by fresh woodpecker feeding. (Right) Woodpecker feeding is often evident higher up in EAB-infested trees.

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

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

Spathius agrili

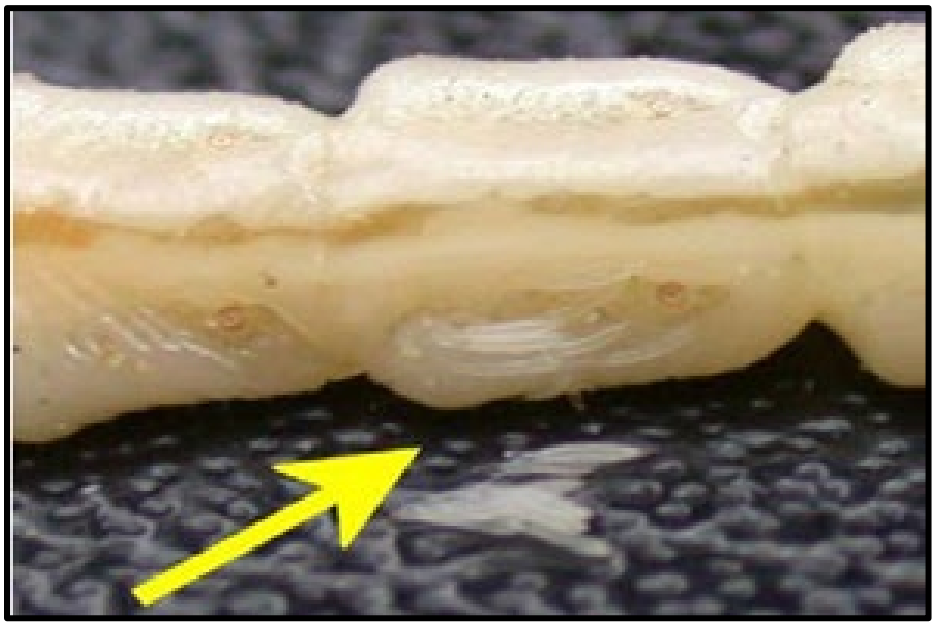


Figure B1. *Spathius agrili* eggs, indicated by an arrow, laid on the surface of an EAB larva.

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

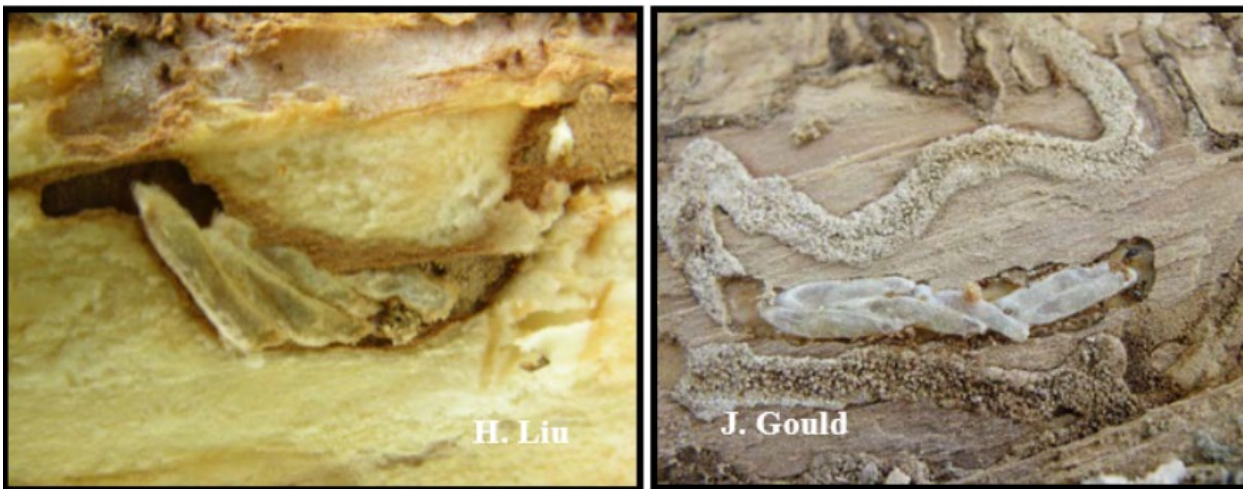


Figure B3. Two photos of silken cocoons of *Spathius agrili* in the host gallery of EAB. The cocoons contain mature larvae or pupae of *S. agrili*.

Figure B4. Female *Spathius agrili* laying eggs through ash bark onto an EAB larva.

Spathius galinae

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

Figure B6. *Spathius galinae* adult female.

Tetrastichus planipennisi

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

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

ha

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

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

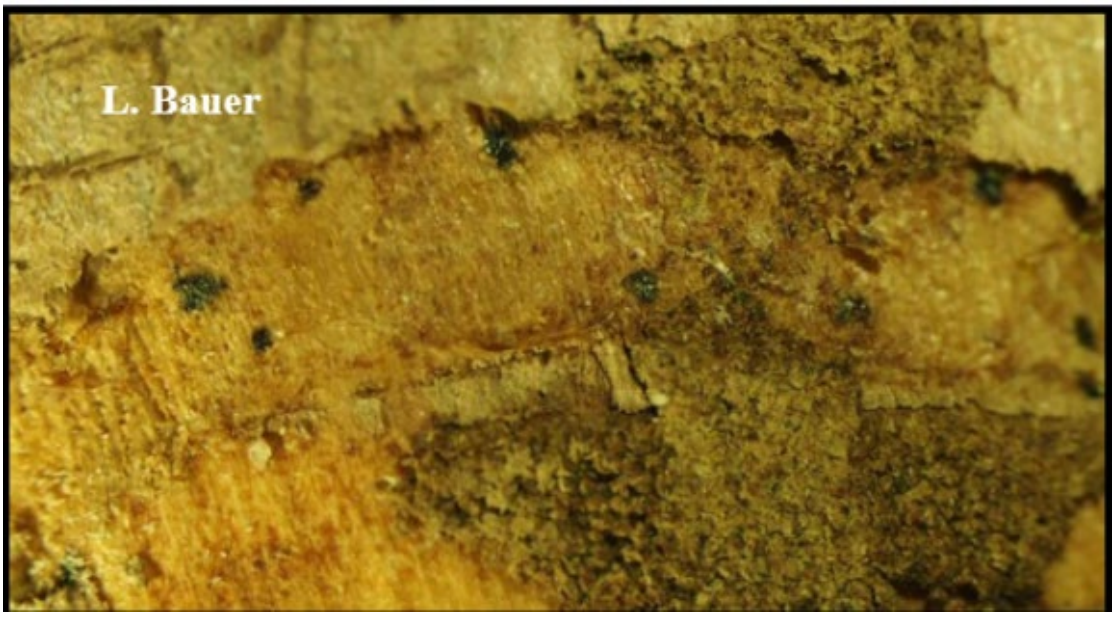


Figure B11. *Tetrastichus planipennisi* meconia (waste) in an EAB gallery. The meconia appear as black spots in the empty EAB gallery after adult emergence is complete.

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

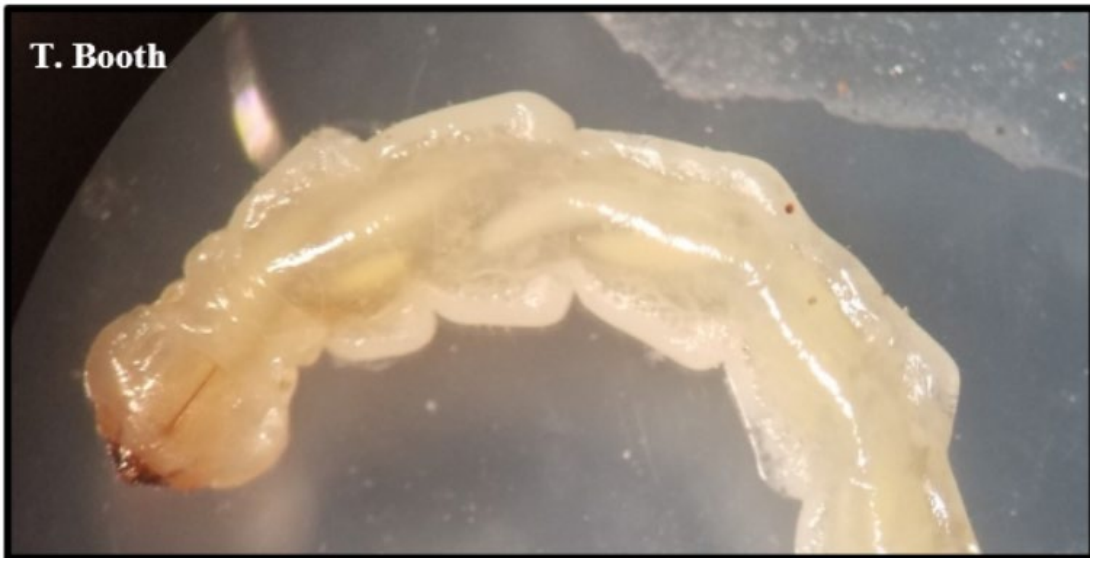


Figure B13. *Tetrastichus planipennisi* larvae are visible swimming within the EAB hemolymph under the microscope.

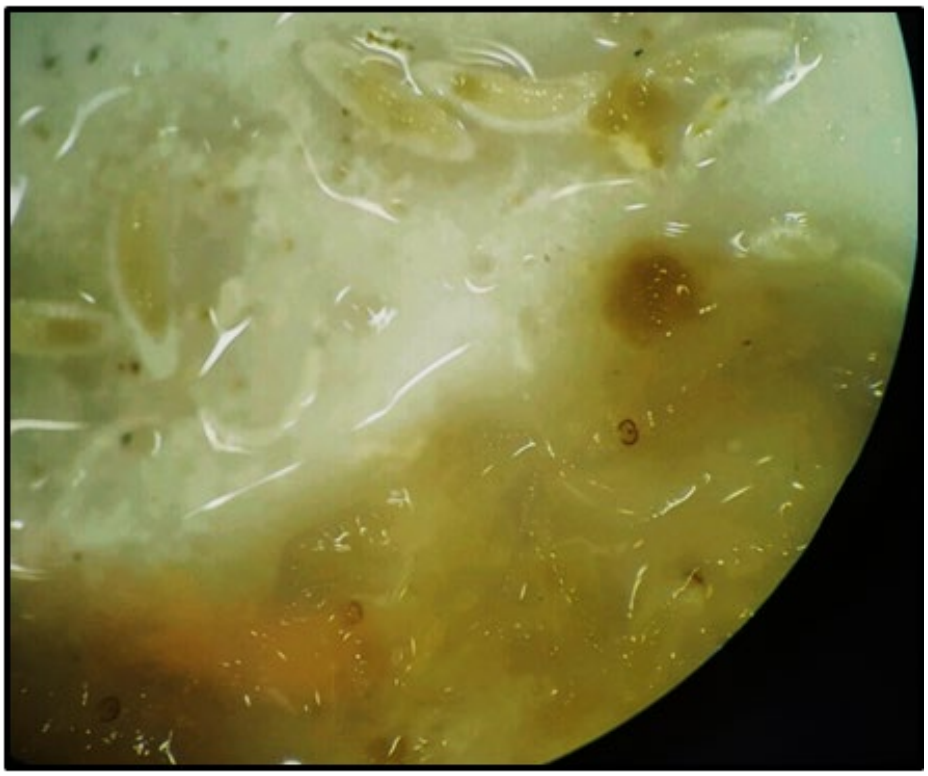


Figure B14. Dissected EAB larva with visible *Tetrastichus planipennisi* larvae under the scope. Use caution when searching for parasitoid larvae or eggs as dissected EAB larvae may have bits and particles that look superficially like parasitoid larvae or eggs.

Oobius agrili

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

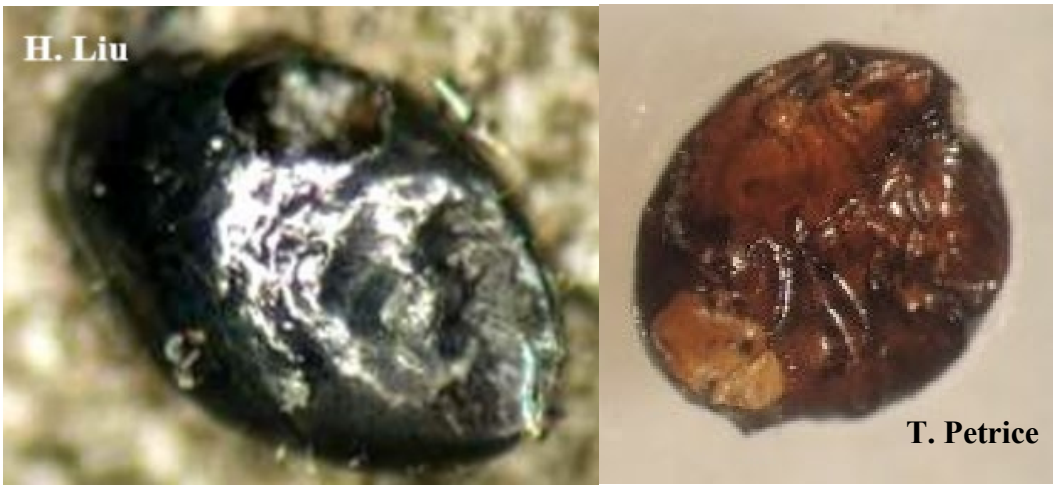


Figure B16. Two EAB eggs, each showing an *Oobius agrili* exit hole. Adult *O. agrili* chew a circular hole through the EAB eggshell and emerge.

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

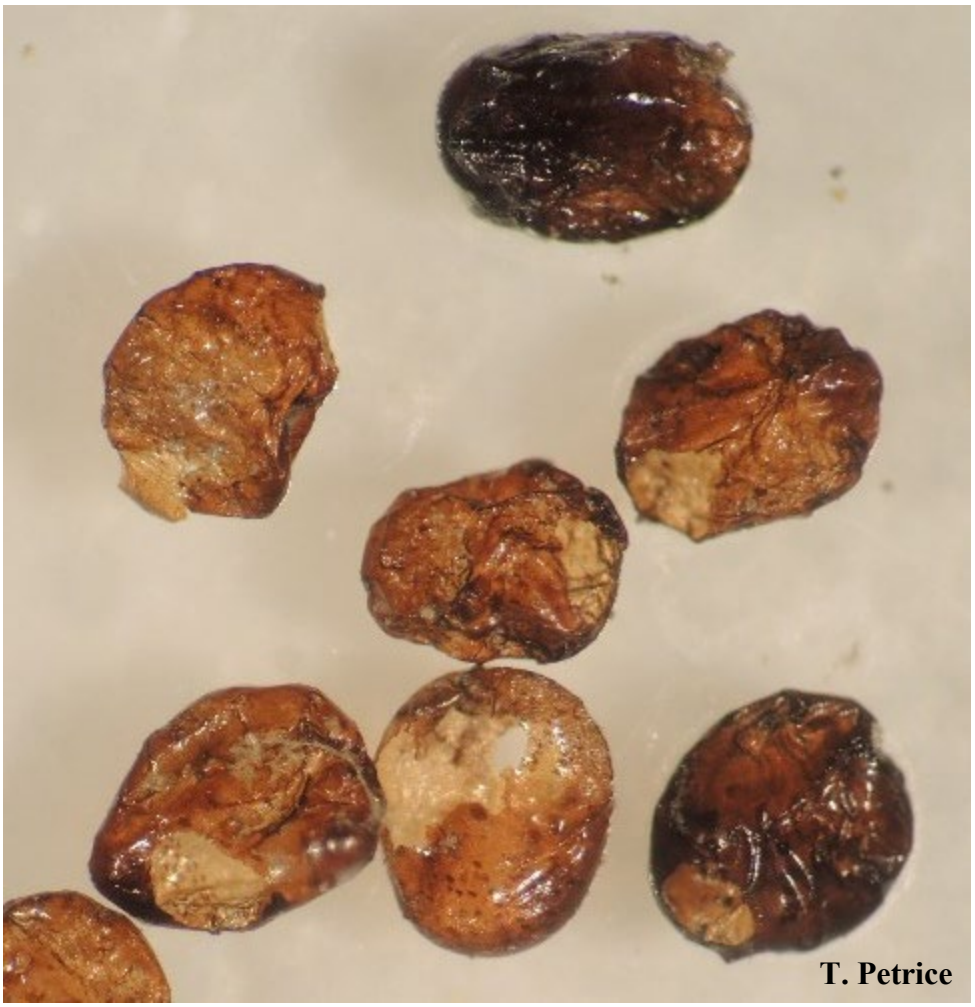
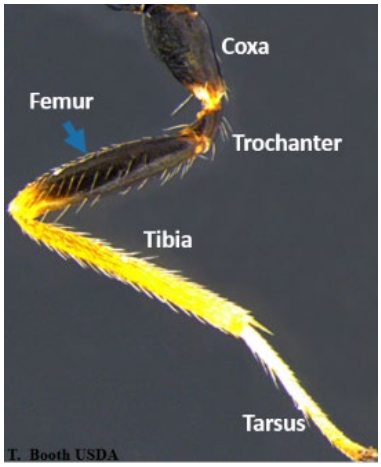


Figure B18. EAB eggs parasitized by *Oobius agrili* and collected from the field. Eggs collected from the field are often less dark in appearance than parasitized eggs produced under laboratory conditions.

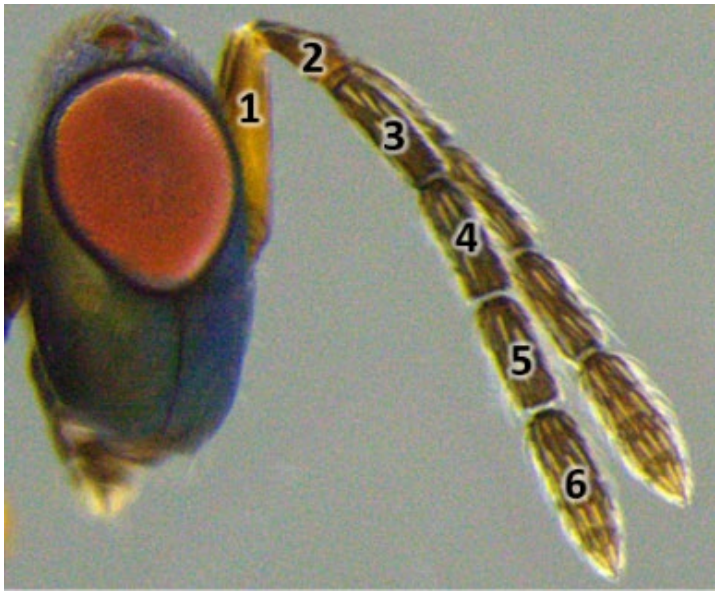
Tetrastichus planipennisi female checklist

Tetrastichus planipennisi females can be identified by five characteristics: 1) dark femurs, 2) 6-segmented antennae, 3) abdomen that is longer than the thorax, 4) last segment of abdomen that is 4x as long as it is wide at the base, 5) smooth thorax with two lateral grooves on the middle thoracic segment. Depending on the condition of your specimen, some characteristics may be hard to see.

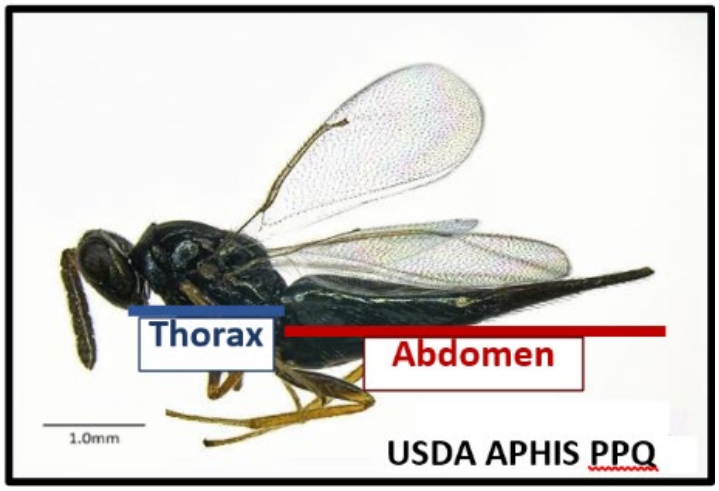
1) **Dark femurs (see arrows).**



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

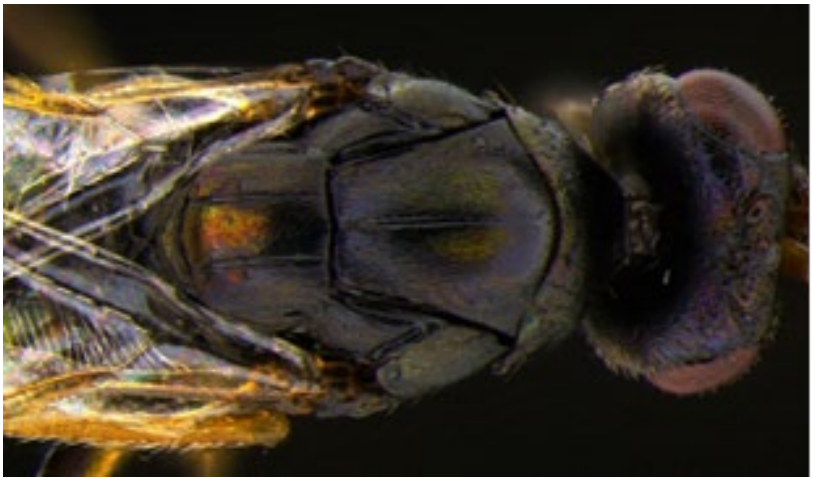


3) Abdomen longer than thorax.



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

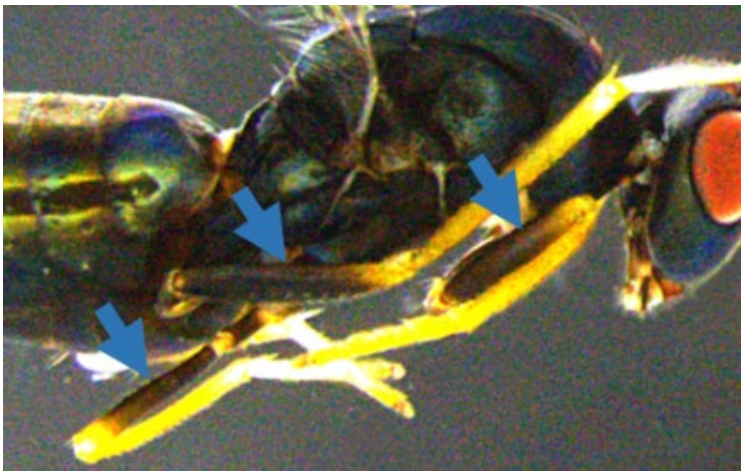
5) Smooth thorax with two lateral grooves on the middle thoracic segment.

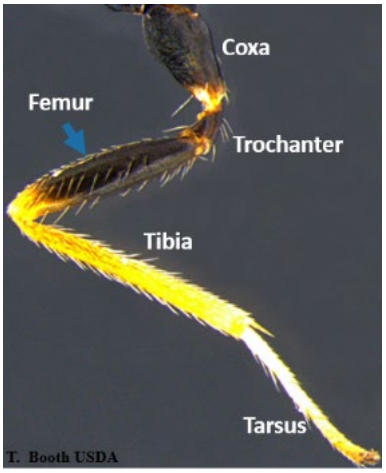


Tetrastichus planipennisi male checklist

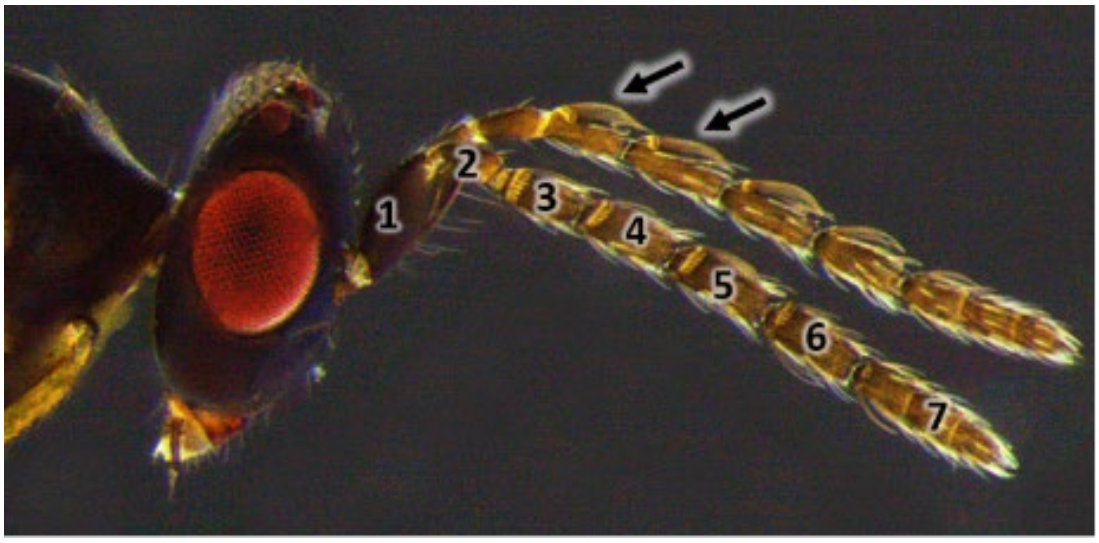
Tetrastichus planipennisi males can be identified by four characteristics: 1) dark femurs, 2) 7-segmented antennae with hairs that look like 'eye lashes', 3) last segment of abdomen not elongated, 4) smooth thorax with two lateral grooves on the middle thoracic segment. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

1) **Dark femurs (see arrows).**

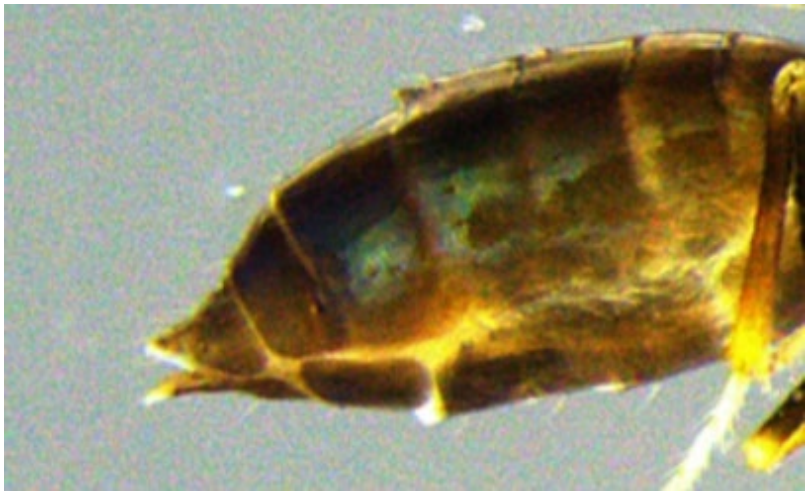




2) **7-segment antennae: scape (1), pedicel (2), four funicles (3-6) and clava (7); clusters of 'eyelash' hairs (see arrows).**



3) **Last segment of abdomen not elongated.** Note that the aedeagus may be visible; not to be confused with an ovipositor.



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

Spathius galinae checklist

Spathius galinae can be identified by the following characteristics: 1)forewings tinged brown with clear spots; wings with brown veins, 2) spherical head and eye; back of the head smooth and without wrinkles, 3) base of the tibia of all legs is yellowish, 4) short pedicel, 5) long antennae with >26 segments, and 6) IF FEMALE: long ovipositor. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

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

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

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

4) **Pedicel (narrow waist connecting the thorax and abdomen) is short.**

5) **Long antennae with >26 segments. Antennae 1.2x length of body.** Note that antennae of preserved specimens may be broken/missing segments.

6) **IF FEMALE: Long ovipositor.**

Spathius agrili checklist

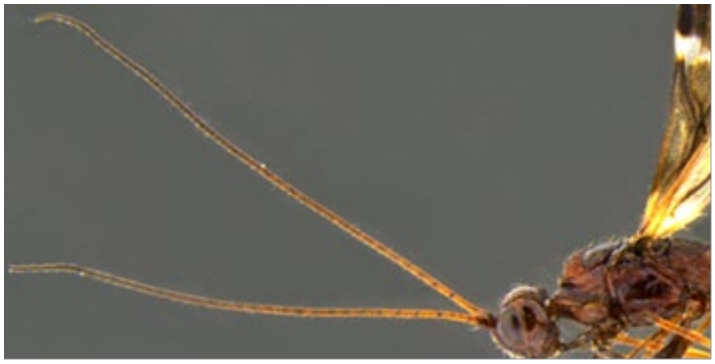
Spathius agrili can be identified by the following characteristics: 1) forewings-tinged brown with clear spots; wings with brown veins, 2) spherical head and spherical eye; back of the head smooth and without wrinkles, 3) short pedicel, 4) long antennae with >26 segments, 1.2 x length of body, and 5) IF FEMALE: Long ovipositor. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

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

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

3) **Pedicel (narrow waist connecting the thorax and abdomen) is short.**

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



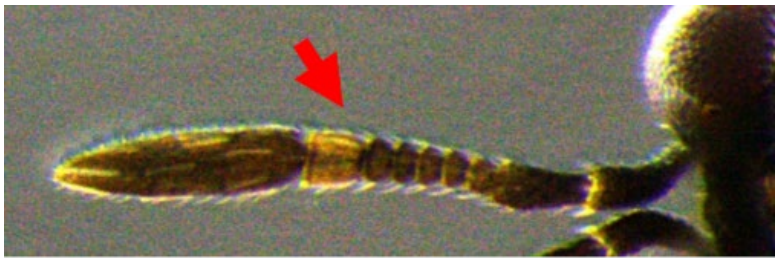
5) **Abdomen very dark in color. IF FEMALE: Long ovipositor**

Oobius agrili checklist

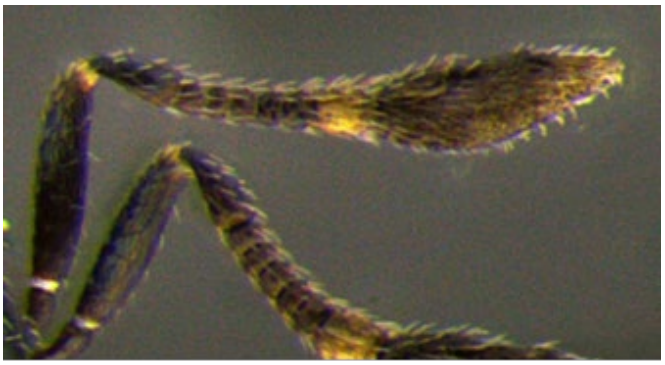
Oobius agrili can be identified by three characteristics: 1) stout body, no waist, 2) second to last antennal segment is light, all other segments are dark, 3) clava (final, club-shaped segment of antenna) is tapered upwards to a blunt point when the insect is viewed in profile. Depending on the condition of your specimen, some characteristics may be hard to see or confirm.

1) **Stout body, no waist.**

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



3) **Clava tapers upward to a blunt point when insect is viewed in profile.**



Appendix D – Releasing parasitoids for optimal establishment

Spathius agrili is expected to establish in areas where EAB undergoes a one-year life cycle, while *Tetrastichus planipennisi* and *S. galinae* are expected to establish in areas where EAB has a two-year life cycle. We collected EAB overwintering data 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 late-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 GDD50F accumulate in the summer, and we do not recommend releasing this species in these locations.
- We will follow the same guidelines used for *T. planipennisi* for releasing *Spathius galinae*.
- *Spathius agrili* will only be released at sites that accumulate > 3,500 GDD50F. It has not established in areas with fewer GDD.

Appendix D – Releasing parasitoids for optimal establishment

Table D1. Maximum threshold for accumulated GDD50F for January 1 – September 30 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	GDD Threshold	Percentage of sites samples with *T. planipennisi* establishment
51-80%	2,985	92%
26-50%	3,500	78%
11-25%	3,975	50%
0-10%	-	23%

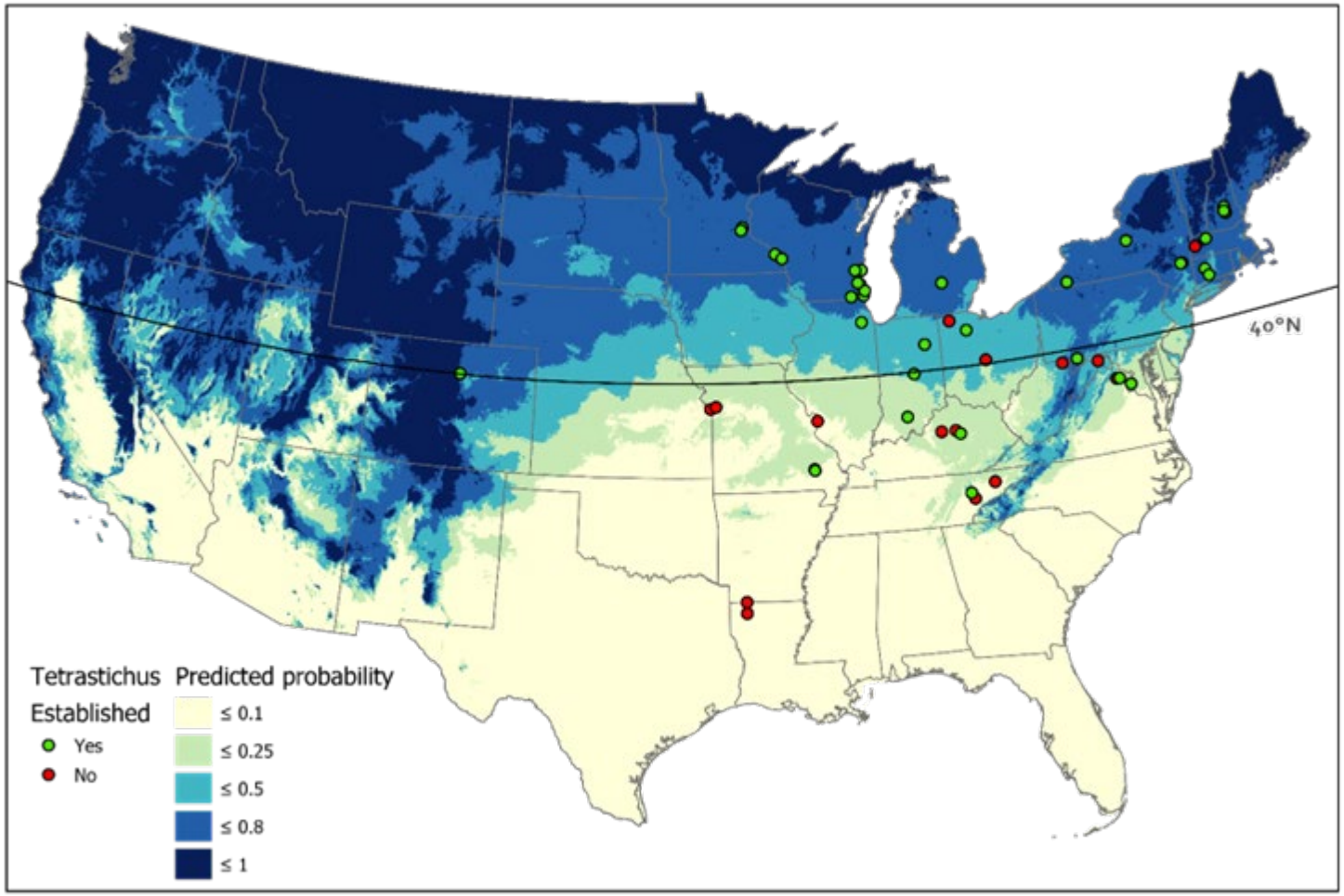


Figure D1. Predicted proportion of EAB that spend the winter as larvae instar 1-4, not as J-larvae. Locations where *Tetrastichus planipennisi* has been collected two or more years following the final release are indicated in green. Locations where samples were collected but *T. planipennisi* was not recovered are marked in red.

Appendix E – Crown condition of ash trees

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

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

Native and Naturalized Parasitoids of EAB

You may come across native or naturalized parasitoids of EAB when you are sorting through your yellow pan traps or peeling trees. You are not required to record or ship these parasitoids, but you may be interested to know they are present at your site.

Figure F1. *Atanycolus* spp. Large native parasitoids with a distinct red or orange abdomen. In contrast, the head and thorax are jet black. The wings are dark grey. Photo by J. Duan.

Figure F2. *Balcha indica*. Naturalized in the eastern U.S. Native to Southeast Asia. Solitary ectoparasitoid of EAB. Pronotum textured. Dark brown with copper, blue, green, and purple lusters under different angles of light. Photo by J. Hansen.

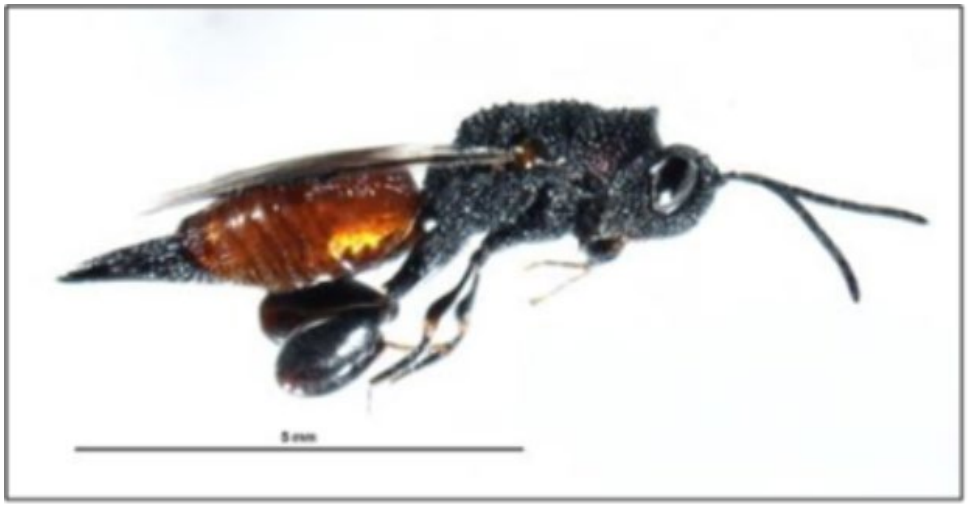


Figure F3. *Phasgonophora sulcata*. A native solitary endoparasitoid. Distinct red/orange section on the abdomen. Thorax is black and pitted. Hind leg femur is swollen with teeth. Photo by J. Duan.

Life stages of native parasitoids

Atanycolus spp.

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

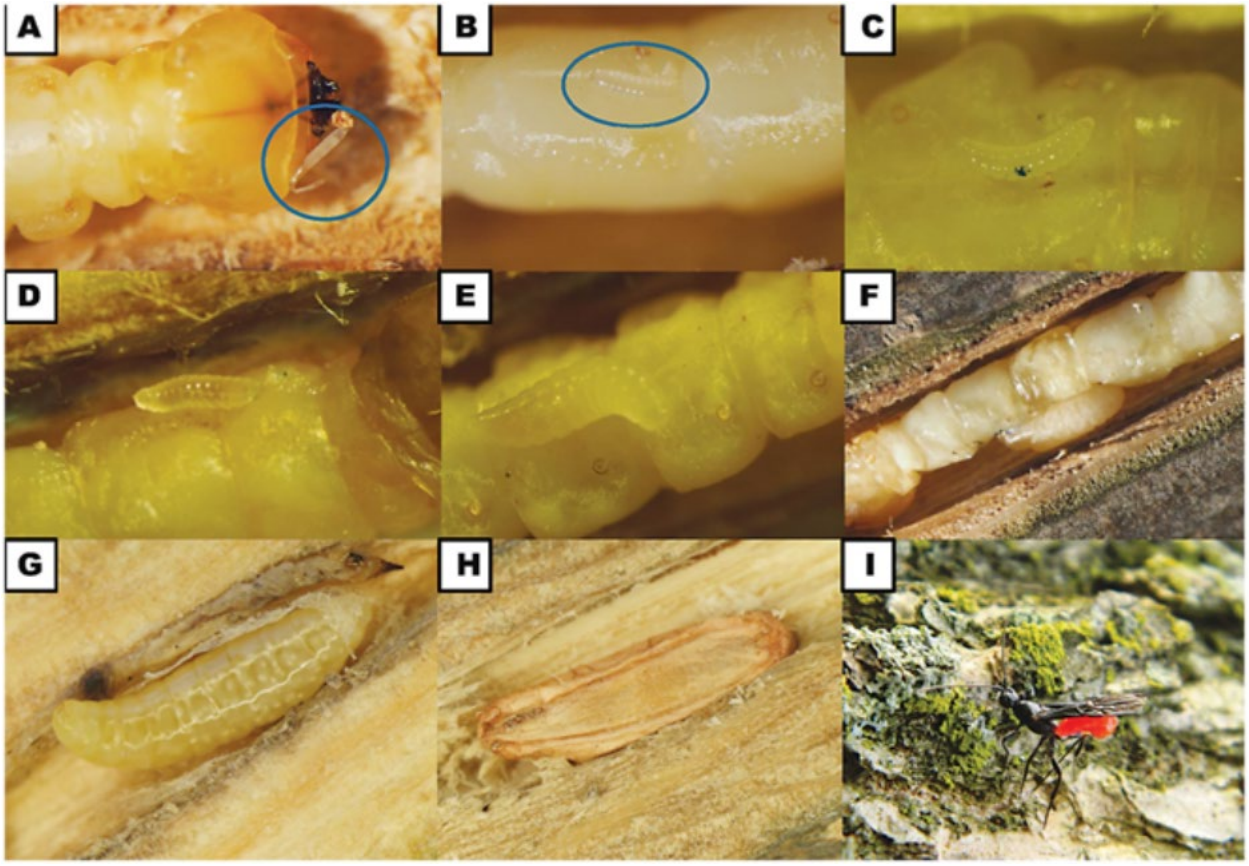


Figure F4. Stages of *Atanycolus cappaerti*. (A) Egg on an EAB larva at 3× magnification; (B) 1st instar at 3× magnification; (C) 2nd instar at 3× magnification; (D) 3rd instar at 3× magnification; (E) 4th instar at 3× magnification; (F) 5th instar at 1.8× magnification; (G) 6th instar at 1.8× magnification; (H) pupal cocoon at 1.8× magnification; (I) Eclosed adult.

From: Duan, J. J., & Schmude, J. (2016). Biology and life history of *Atanycolus cappaerti* (Hymenoptera: Braconidae), a North American larval parasitoid attacking the invasive emerald ash borer (Coleoptera: Buprestidae). *Florida Entomologist*, *99*(4), 722-729. https://doi.org/10.1007/s10526-011-9408-0

Balcha indica

This species is native to southeast Asia but has naturalized in the eastern US. It is a solitary ectoparasitoid of EAB.

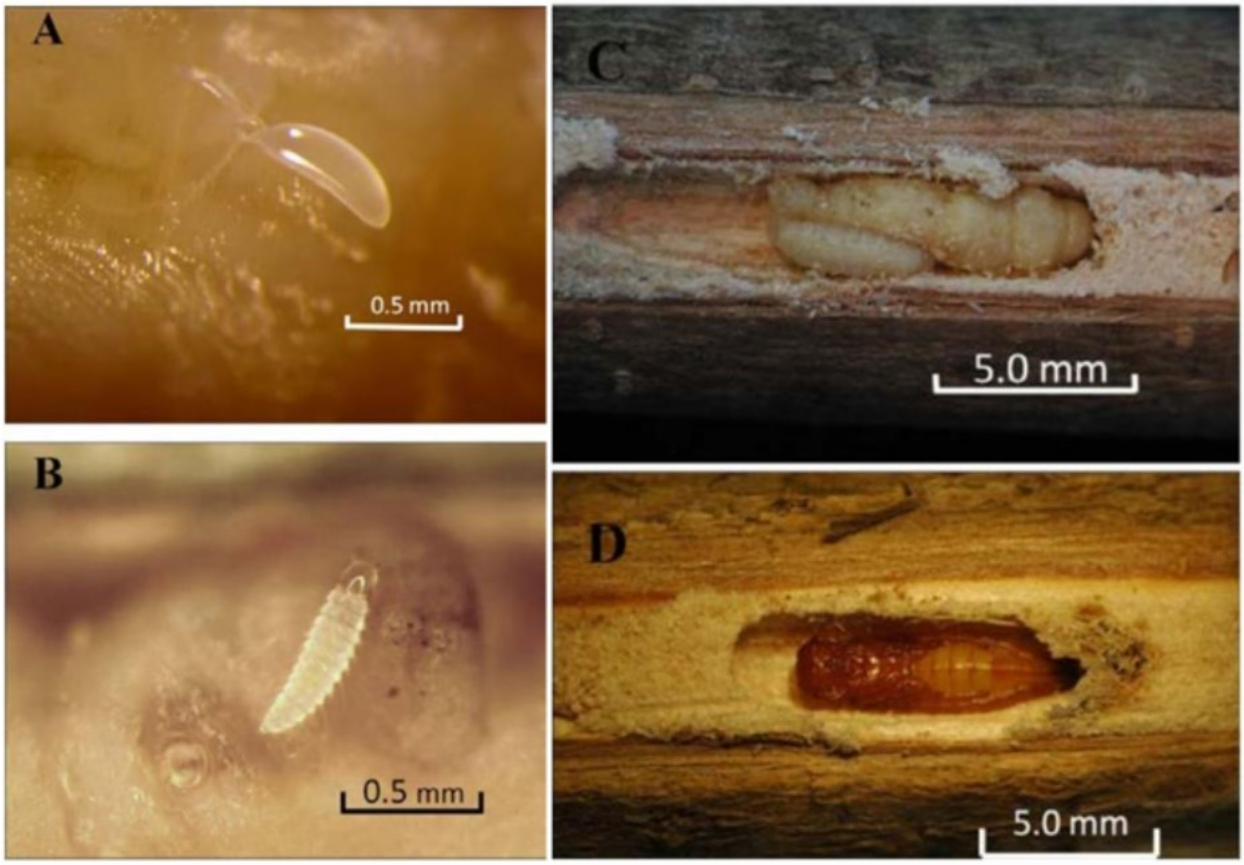


Figure F5. Stages of *Balcha indica*. (A) A single egg laid on the surface of an EAB host; (B) 1st instar larva feeding on the EAB pre-pupa; (C) the "maggot-like" intermediate-stage larva parasitizing EAB pupa; (D) pupa in cell.

From: Duan, J. J., Taylor, P. B., & Fuester, R. W. (2011). Biology and life history of *Balcha indica*, an ectoparasitoid attacking the emerald ash borer, *Agrilus planipennis*, in North America. *Journal of Insect Science*, *11*, 127. https://doi.org/10.1673/031.011.12701

Phasgonophora sulcata

Phasgonophora sulcata is a solitary endoparasitoid, so you will only see a single larva (rarely two, but one is usually dead). The larva is usually found near the tail end of the EAB larva. *Phasgonophora sulcata* has a distinctive head end and tail end, unlike a *Tetrastichus planipennisi* larva.

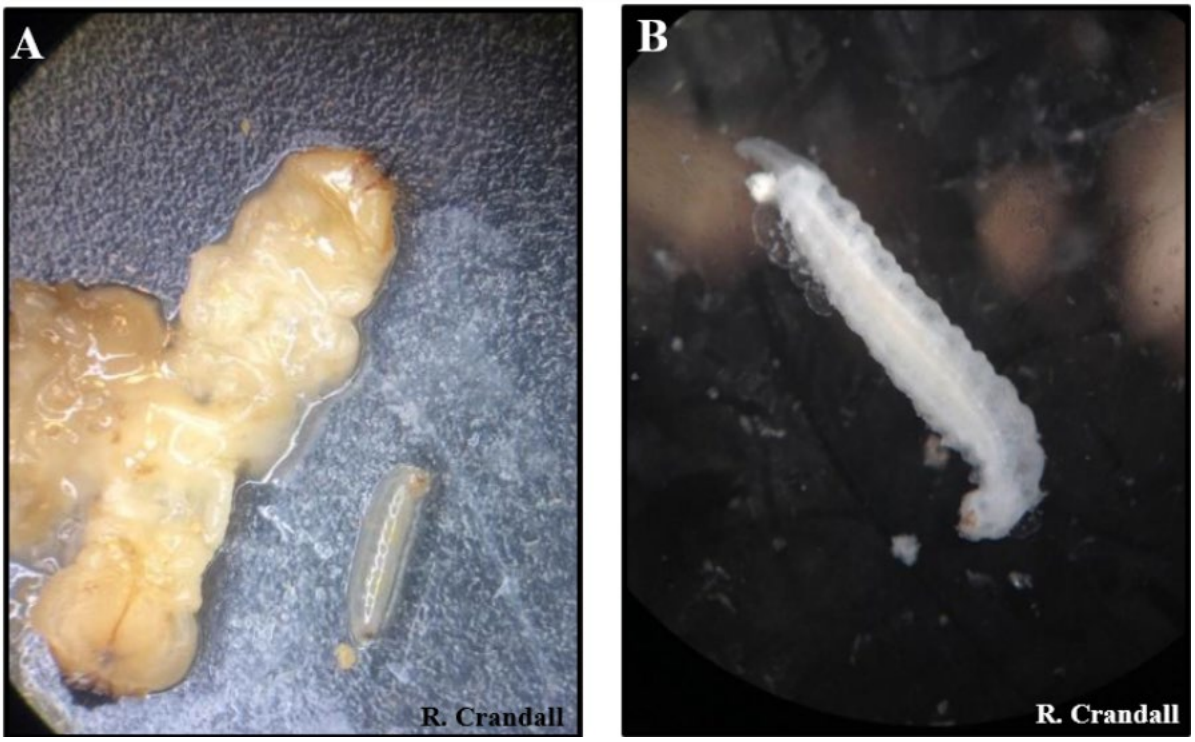


Figure F6. (A) A dissected EAB larva on the left and a *Phasgonophora sulcata* on the right. (B) *Phasgonophora sulcata* larva with the tail at the top and the head at the bottom.

General Links

Bark Sifting for *Oobius agrili*: Biological Control of Emerald Ash Borer: Bark sifting for Oobius agrili

Detailed Instructions for Making Yellow Pan Traps: https://www.aphis.usda.gov/sites/default/files/detailed-instructions-ypt.pdf

Calculating Growing Degree Days: http://uspest.org/cgi-bin/ddmodel.us

Emerald Ash Borer Network: http://www.emeraldashborer.info/

MapBioControl: www.mapbiocontrol.org

USDA APHIS Emerald Ash Borer Home Page: https://www.aphis.usda.gov/plant-pests-diseases/eab

USDA APHIS EAB Program Manual: https://www.aphis.usda.gov/sites/default/files/eab-manual.pdf

USDA APHIS: "Debarking Ash Tree Logs to Look for Emerald Ash Borer" https://www.youtube.com/watch?v=sMV-1r5lnvs&t=9s

Questions and Answers: Biological Control for Emerald Ash Borer https://www.aphis.usda.gov/sites/default/files/faq_eab_biocontrol.pdf

Questions and Answers: Release and Recovery of Biological Control for Emerald Ash Borer https://www.aphis.usda.gov/sites/default/files/qa-eab-release-and-recovery.pdf

Datasheet Templates

Parasitoid Release Datasheet: https://www.aphis.usda.gov/sites/default/files/parasitoid-release-datasheet.xlsx

Tree Peeling Datasheet: https://www.aphis.usda.gov/sites/default/files/tree-peeling-datasheet.xlsx

Yellow Pan Trap Recovery Datasheet: https://www.aphis.usda.gov/sites/default/files/ypt-recovery-datasheet.xlsx

Training Videos – EAB Biocontrol Release and Recovery Guidelines

Module 1 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 7, 2022 - YouTube

Introduction, background, and lifecycle of EAB, how to identify EAB and possible look-a-likes, history of biological control of EAB, how to identify ash and possible look-a-likes, impacts of EAB parasitoids. **(1 hour 40 minutes)**

Module 2 Emerald Ash Borer Release and Recovery Guidelines Workshop – April 14, 2022 - YouTube

Mass rearing of EAB biological control agents for release, choosing sites and considerations for releasing parasitoids, conducting parasitoid releases, and an introduction to MapBioControl. **(1 hour 47 minutes)**

Module 3 Emerald Ash Borer Release and Recovery Guidelines – April 21, 2022 - YouTube

Evaluating parasitoid establishment: debarking for *Oobius agrili*, peeling trees for larval parasitoids, and yellow pan traps. **(2 hours 21 minutes)**