

Emerald Ash Borer Biological Control RELEASE GUIDELINES 2026



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This is a SUBSECTION of the Emerald Ash Borer Biological Control Release and Recovery Guidelines, intended to instruct users on PARASITOID FIELD RELEASE PROTOCOLS.

To access the full document, follow this link: <https://www.aphis.usda.gov/sites/default/files/eab-field-release-guidelines.pdf>

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Cover photographs by: Cup with adult *Spathius galinae* (left): Theresa Booth, USDA APHIS PPQ; Ash bolt with parasitized EAB larvae (center): Theresa Booth, USDA APHIS PPQ; Oobinator (right): USDA APHIS PPQ.

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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.

OTHER PROGRAM CONTACTS

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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 four consecutive years 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:

Site Basics

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

General Details

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!**

FAQs

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.

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 $\leq 25\%$, 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 planipennis*, 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

The screenshot displays the 'mapB' web application interface. At the top left, a fly icon is next to the 'mapB' logo. Below it, a navigation bar contains 'HOME', 'RELEASE' (circled in red), and 'REC'. A 'New Release Site' modal window is open, titled 'Step 3'. It has three main sections: 'Status' with radio buttons for 'Proposed' (selected), 'Approved', 'Deferred', and 'Denied'; 'Creation Date' with a text input field containing '26-Mar-26' and a note 'US style 1.-Abc-11 format'; and 'Location' with dropdowns for 'Country' and 'State(USA)/Province(Canada): *', and text inputs for 'Site Name: *', 'Site Location: *', 'Latitude: *', and 'Longitude: *'. Below these are dropdowns for 'Plot: *' and 'Type: *', and a 'Notes' text area. At the bottom of the modal is a 'General Information (Optional):' section with a 'Size Wooded Area (acres):' input field set to '0' and a 'Create' button. In the background, a 'Release Site' list is visible with a 'New' button circled in red (Step 2). The list has columns for 'ID', 'NAME', and 'STATE' and shows entries 10 through 14. A pagination bar at the bottom of the list shows 'Showing 1 to 5 of 1,493 entries' and page numbers 1, 2, 3, 4.

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 planipennis

- *Tetrastichus planipennis* 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 planipennis* 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 PARASITIDS

Adult Parasitoids in Plastic Cups



A cup with adult *Spathius galinae*. Ash bolt with parasitized EAB hanging from a tree. An Oobinator hanging from a branch.

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).

The image shows three steps in the MapBioControl interface:

- Step 1:** A table titled 'Release Site Management' with columns for ID, NAME, STATE, COUNTY, DATE, and LOCATION. The row with ID 4185 is highlighted in blue.
- Step 2:** The 'Parasitoid Releases' tab is selected in the site management interface.
- Step 3:** A 'New parasitoid release report' form with the following fields:
 - Release:** Release Date (30-Mar-26), Release Time (11:11 AA format).
 - Weather:** Conditions (dropdown), Wind Speed (dropdown), Temperature (°F) (text input).
 - Oobius Released:** Count of Female (0), Stage (dropdown).
 - Spathius agrilii Released:** Count of Female (0), Stage (dropdown).
 - Spathius gallinae Released:** (empty form).

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**

Appendix A - EAB life stages and damage

EAB life stages

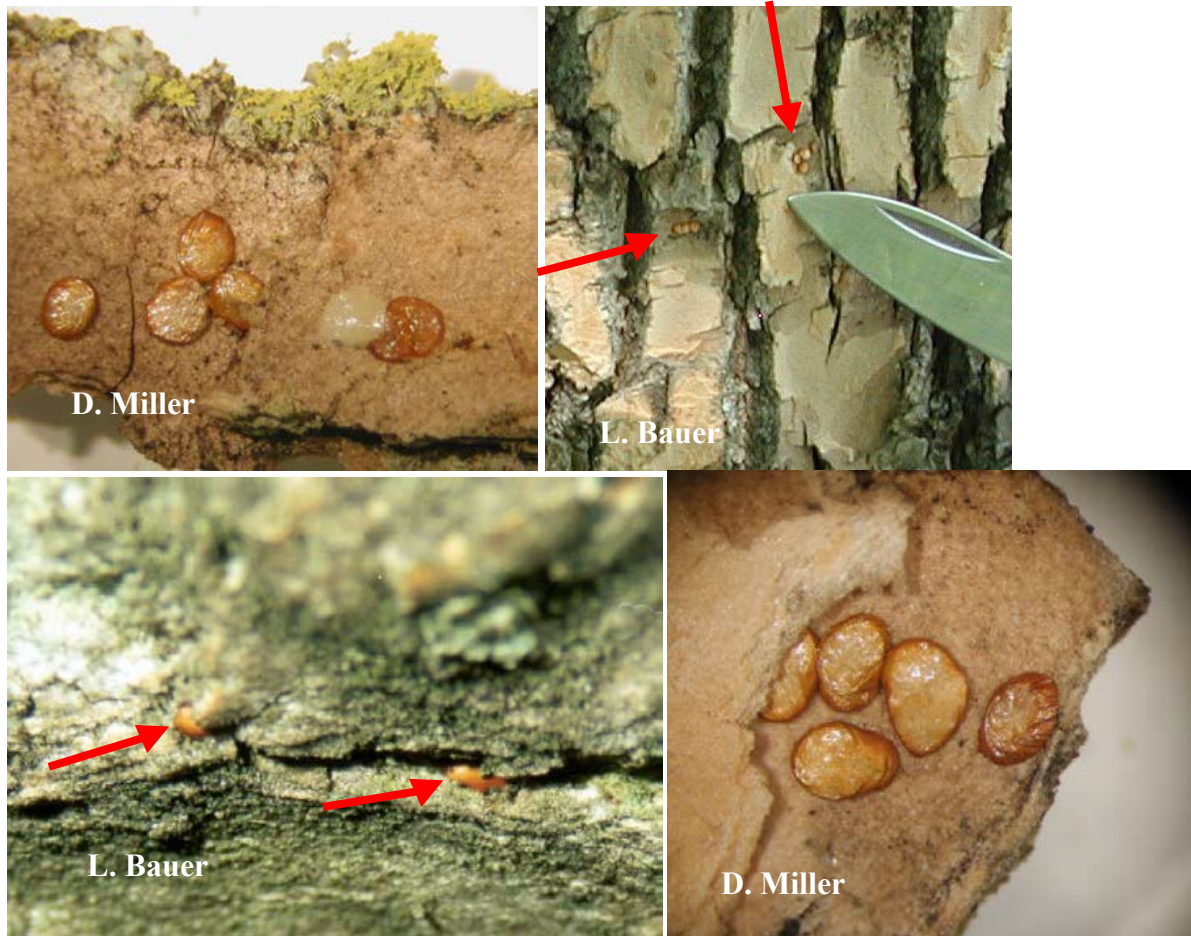


Figure A1. EAB eggs. (Top left) The white egg is newly laid, while the other eggs have matured to an amber color. (Top right) Clusters of eggs laid between bark flakes that have been exposed with a knife. Arrows indicate the eggs. (Bottom left) Single eggs laid in bark crevices. Arrows indicate the eggs.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

Appendix A - EAB life stages and damage

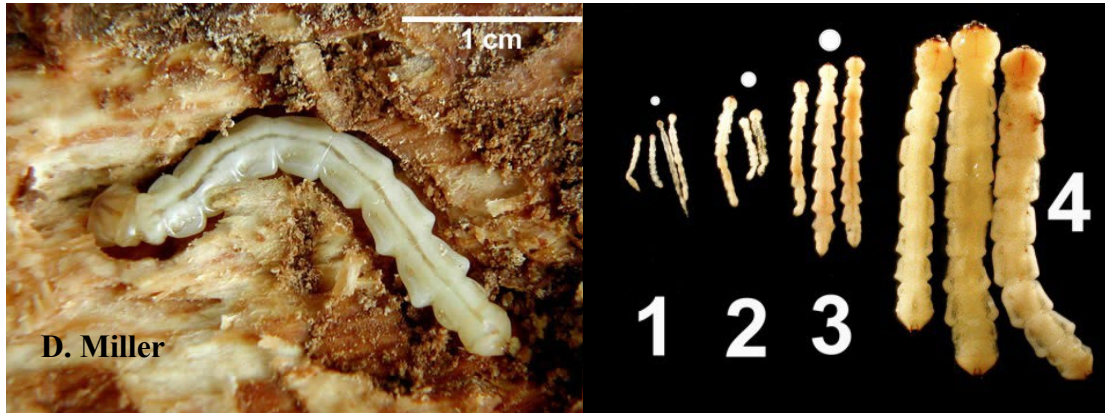


Figure A2. EAB larvae. (Left) Mature 4th instar EAB in a gallery. (Right) The four instars of EAB larvae.



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.

Appendix A - EAB life stages and damage



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

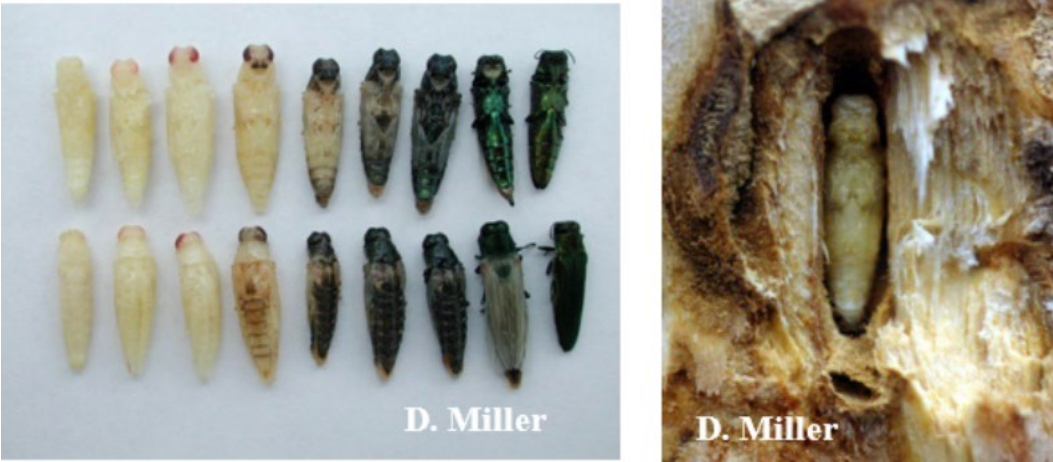


Figure A6. EAB pupae. (Left) Stages of maturing pupae from left to right. (Right) Pupa in outer sapwood.

Appendix A - EAB life stages and damage



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.

Appendix A - EAB life stages and damage

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.

Appendix A - EAB life stages and damage



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.

Appendix A - EAB life stages and damage

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.



Appendix A - EAB life stages and damage



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.

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

Appendix D – Releasing parasitoids for optimal establishment

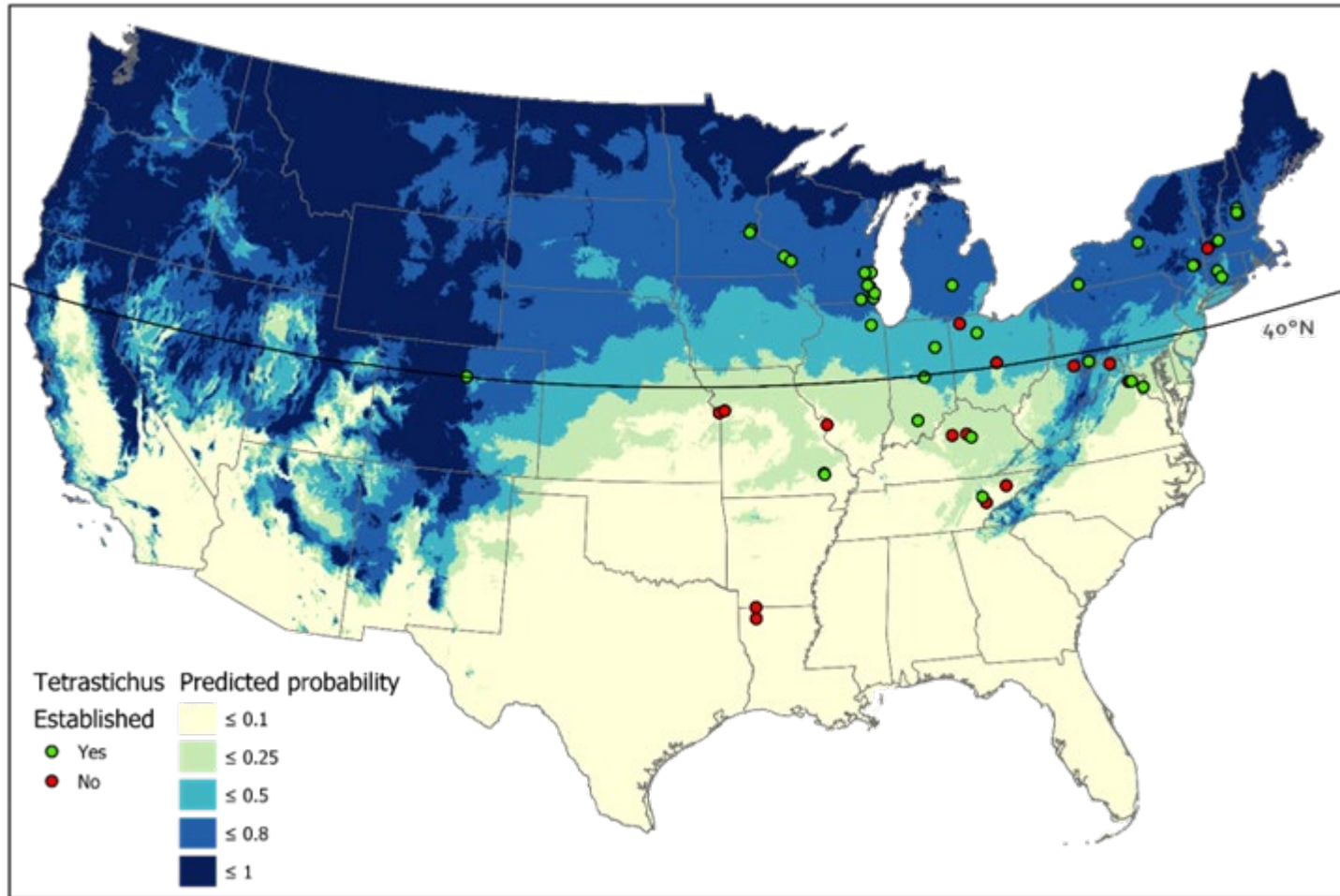


Figure D1. Predicted proportion of EAB that spend the winter as larvae instar 1-4, not as J-larvae. Locations where *Tetrastichus planipennis* has been collected two or more years following the final release are indicated in green. Locations where samples were collected but *T. planipennis* was not recovered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

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.

