Emerald Ash Borer
Biological Control
Release and Recovery
Guidelines
Emerald Ash Borer, *Agrilus planipennis* (Fairmaire), Biological Control Release and Recovery Guidelines – May 2013

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INTRODUCTION

BRIEF HISTORY OF EAB IN NORTH AMERICA

Emerald ash borer (EAB), a beetle from Asia that feeds on ash trees, was discovered as the cause of extensive ash mortality in southeast Michigan and adjacent areas of Canada in 2002. It is thought that this destructive pest was introduced in the early 1990’s in infested solid wood packing material originating in Asia.

Shortly after EAB was discovered in North America, federal and state regulatory agencies placed infested counties under quarantine and eradication activities were initiated. Due to the magnitude of the EAB infestation in North America, the potential for natural and artificial dispersal of EAB, limited EAB detection and control methods, and high costs, program objectives shifted away from eradication to containment and management of the pest. By April 2013, EAB infestations were known in nineteen states (Indiana, Illinois, Kentucky, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Virginia, West Virginia, Wisconsin, Iowa, Tennessee, Kansas, Connecticut, Massachusetts, and New Hampshire) and two Canadian provinces (Ontario and Quebec). At present, the most promising long-term approach for reducing EAB populations and conserving ash in forested areas of North America is biological control.

LIFE-CYCLE OF EAB

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

EAB adults begin to emerge from the trunks of ash trees after the accumulation of 400-500 growing degree days base 50°F (GDD). Peak adult activity occurs at ~1000 GDD. After emergence, adults fly into the ash canopy where they feed on leaves throughout their lives. EAB adults start mating one week after emergence, and females begin laying eggs 2-3 weeks later. In the field, EAB adults are readily observed mating and egg-laying on the trunks of 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 the major branches. Eggs are laid in areas where the bark is rough, and between bark layers or in bark crevices. Eggs are approximately 1.0 mm long x 0.6 mm wide and creamy white when laid; fertile eggs gradually turn amber after a few days (Appendix A). The eggs hatch after about two weeks.

**Larvae**

Newly hatched larvae bore through the bark to the phloem and outer layer of new sapwood where they feed until the weather gets too cold in the fall. There are four stages of larval development (instars) (Appendix A). As they feed, the larvae create long serpentine galleries filled with frass, which enlarge in width as they grow (Appendix A). Larvae are creamy white, and dorso-ventrally flattened (Appendix A). When fully mature, fourth-instar larvae are 26 to 32 mm long. Their 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 pair of small brown structures called urogomphi, which are characteristic of all larvae in the genus *Agrilus* (Appendix A).

**Overwintering larvae, prepupae, pupae, and adults**

In the fall, mature fourth-instar EAB larvae excavate pupal chambers in the sapwood or outer bark where they fold into overwintering “J-shaped larvae” (Appendix A). In the spring, the J-shaped larvae shorten into prepupae then shed their cuticle to become naked pupae. Pupae are initially creamy white, but the eyes turn red and the body begins to darken as they develop (Appendix A). To emerge from ash trees, adults chew D-shaped exit holes (Appendix A) through the bark and are capable of immediate flight upon emergence. EAB larvae that are immature as cold weather arrives in the fall will simply overwinter in their larval gallery. Mature larvae complete development (i.e. become an adult beetle) the following spring, whereas younger larvae may require another summer of feeding to complete development.

**DAMAGE AND SIGNS OF INFESTATION**

EAB larvae damage ash trees by feeding on the phloem. In a new infestation, when just a few EAB larvae infest a tree, the tree responds by forming scar tissue or “callous” around EAB galleries, and the tree may show few outward signs of infestation. On some trees or branches, however, the callous may cause the bark to split, exposing the EAB gallery beneath (Appendix A). As EAB larval density increases, the movement of nutrients through the phloem is disrupted and evidence of tree stress increases such as yellow foliage on dying branches, dead branches, small leaves, thinning crowns, and epicormic shoots (Appendix A).
Woodpeckers feed on EAB larvae living under the bark of trees. We have found woodpecker feeding is one of the best indicators of early EAB infestation with the most obvious symptoms including bark scaling (removal of bark flakes) and feeding holes through the bark (Appendix A). Although difficult to detect, especially high in the canopy, the D-shaped exit holes chewed by emerging adults are diagnostic indicators of EAB infestation (Appendix A).

ECONOMIC CONSEQUENCES OF EAB INFESTATIONS

The cost of managing EAB is already high. On average, federal and state resource managers spend $29.5 million per year to manage EAB populations. The compensatory value of the 8 billion ash trees in U.S. timberland potentially infested with EAB is $282 billion. States in the eastern U.S. produce nearly 114 million board feet of ash saw timber annually, with a value of $25.1 billion. White, black, and green ash make up >7 percent of the hardwood stand mix and 5.5 percent of the total stand mix (including conifers) in the northeastern United States and eastern Canada. The wood is used for a variety of applications including tool handles, baseball bats, furniture, cabinetry, basketry, solid wood packing materials, pulp, and paper. The continued spread of EAB threatens our ash resources and will permanently alter ecosystems in the eastern states. The 16 native species of ash, some with limited distributions in North America, are now threatened by EAB.

In addition to its value to the timber industry and the forest ecosystem, ash is one of the most popular landscape trees because of its tolerance of a range of environmental conditions and resistance to other pests. Ash was the most commonly planted tree species used to replace elm trees decimated throughout North America by Dutch elm disease and for new residential
and commercial developments. The estimated cost of treating, removing, and replacing 37.9 million ash trees in urban and residential settings in 25 states is $25 billion. Nationwide, the nursery industry produced an estimated 2 million ash trees each year. With median approximate values ranging from $50 to $70 per tree, the annual ash nursery stock was worth between $100 and $140 million.

HOST RANGE OF EAB

In eastern North America, EAB attacks ash species in the genus *Fraxinus*, including but not limited to green ash (*F. pennsylvanica*), white ash (*F. americana*), black ash (*F. nigra*), pumpkin ash, (*F. profunda*), and blue ash (*F. quadrangulata*). In China, native ash species, including *F. chinensis* and *F. mandshurica*, are less susceptible to EAB than North American species commonly planted in China such as velvet ash (*F. velutina*) and green ash.
BIOLOGICAL CONTROL OF EMERALD ASH BORER

Biological control (or biocontrol) is the practice of importing and releasing natural enemies from a pest’s native range to control populations in the area of introduction. Biocontrol has been used for over 100 years in the U.S. and has successfully controlled invasive plant and insect pests such as gypsy moth, winter moth, ash whitefly, eucalyptus longhorned borer, purple loosestrife, and Klamath weed. Because EAB is from northeast Asia, U.S. and Chinese scientists have been searching for EAB and its natural enemies in that region since 2003. In Asia, EAB population densities are relatively low due to the combined effects of EAB-resistance in Asian ash species, scarcity and patchiness of forests, and the EAB natural enemy complex. Exploration for EAB natural enemies in China by USDA and Chinese researchers yielded several hymenopteran parasitoids. Three of these parasitoids have been approved for release as biological control agents of EAB in the U.S.

NATIVE NATURAL ENEMIES

In southeast Michigan, <1% EAB larval parasitism was found by researchers surveying for potential natural enemies of EAB in 2003 and 2004. Parasitoid species reared from ~3,000 EAB larvae included several native hymenopteran species: *Phasgonophora sulcata* (Chalcididae), *Spathius floridanus* (Braconidae), *Atanycolus* spp. (Braconidae), and one exotic parasitoid *Balcha indica* (Hymenoptera: Eupelmidae). No native egg parasitoids have been reared from EAB eggs. More recently, *Atanycolus* spp. were found parasitizing up to ~70% EAB larvae locally in Michigan, but overall populations of EAB are not controlled by native natural enemies. The development of a biological control program for EAB management using non-native parasitoids continues to progress because parasitism of EAB
in the U.S. is low compared to that reported for our native \textit{Agrilus} spp. and for EAB in
China.

**BIOLOGY OF EAB PARASITOIDS**

\textit{Oobius agrili} parasitizes up to 60\% of EAB eggs laid during the summer in some areas of
China. Tiny female \textit{Oobius} accomplish this by searching the bark of ash trees for EAB eggs,
which are laid in bark crevices and between layers of bark. When \textit{Oobius} finds an EAB egg,
it injects its own egg inside (Appendix B) where it will hatch, grow, and kill the host egg. An
\textit{Oobius} adult will emerge and repeat the cycle for at least two generations during the EAB
egg-laying season. Each \textit{Oobius} adult parasitizes an average of \(~80\) EAB eggs during its
lifetime. \textit{Oobius} spend the winter as larvae inside EAB eggs and emerge as adults the
following spring.

\textit{Spathius agrili} parasitizes up to 90\% of EAB larvae in ash trees in some parts of China.
Female \textit{Spathius} parasitize EAB larvae by drilling through the bark (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, causing its death. The cycle is repeated 1-2 times each
summer and fall depending on climate. \textit{Spathius} overwinter as larvae or pupae in the host
gallery. Mature larvae spin silken cocoons in which they pupate and emerge as adults during
the summer.

\textit{Tetrastichus planipennisi} is another larval parasitoid of EAB from China, where it attacks
and parasitizes up to 50\% of EAB larvae in some areas. The life cycle of \textit{Tetrastichus} is
similar to that of \textit{Spathius}, however, the female parasitoid lays eggs \textit{inside} EAB larvae where
the parasitoid larvae grow, eventually killing their host. \textit{Tetrastichus} completes several
generations each year, and one EAB larva can produce >130 *Tetrastichus* adults. They survive the winter as larvae inside their host or host gallery under the bark of ash trees (Appendix B).

**REARING EAB PARASITOIDS**

The USDA APHIS PPQ Biological Control Production Facility in Brighton, MI was designed to produce EAB parasitoids for field release. These small parasitic wasps must be reared in EAB eggs or larvae, which are produced or harvested from bolts of ash trees felled in nearby woodlots. Although the parasitoids are reared and stockpiled throughout the year for release during the field season, the rearing methods are time and labor intensive. At the present time, production of EAB eggs and larvae limits the number of parasitoids that can be produced. Thus, demand for biological control agents may exceed production for the foreseeable future.

The EAB egg parasitoid, *Oobius*, is reared in eggs laid by EAB adults. Initially, the adult beetles are reared from infested ash trees, which were harvested and refrigerated the previous winter or early spring. After emergence from ash logs, EAB adults are fed greenhouse-grown or field-collected ash leaves throughout their lives. In the field, EAB females oviposit on the bark of ash trees, but in the laboratory, they will deposit their eggs on paper. EAB eggs attached to paper are then exposed to *Oobius* females, which parasitize the eggs. *Oobius* will be shipped in plastic cups as adults or as pupae in EAB eggs on paper.

The two species of EAB larval parasitoid, *Spathius* and *Tetrastichus*, are reared in small-diameter ash bolts in which EAB larvae are grown or inserted under the bark. These infested ash bolts are exposed to either *Spathius* or *Tetrastichus* adults, which detect and parasitize
EAB larvae feeding under the bark. Most of the EAB larvae used to produce larval parasitoids are now produced in the laboratory by applying EAB eggs to live ash logs in which the EAB larvae grow until mature. Parasitoids are then placed with these logs, and parasitism will occur readily.

In the past, adult parasitoids were shipped and released; however, we will begin shipping ash bolts containing *Spathius* or *Tetrastichus* pupae in 2013. This will change how parasitoids are released into the field: ash bolts containing parasitoid pupae will be taken to the field sites and attached to release trees where the parasitoids will emerge naturally under field conditions. Following the evaluation in 2013/2014, shipment of ash bolts containing parasitoid pupae will potentially become the standard method of delivery.

**PROJECT STATUS**

In January 2009, a Biological Control Production Facility became operational in Brighton, MI. As of February 2013, this facility has reared and released over 720,000 EAB parasitoids in fourteen states. These releases will continue while scientists continue to study the establishment, dispersal, and impact these natural enemies have on suppressing EAB populations and the recovery of ash trees. Scientists will also continue to explore the U.S. and Asia for additional EAB natural enemies for possible use in the EAB biological control program. Most recently, a new species of *Spathius* was discovered attacking EAB larvae in Korea and Russia, where the climate is different than in Tianjin, China, where *S. agrili* was collected. Host specificity testing is complete for *Spathius galinae*, and an application for a release permit has been submitted.
FIELD RELEASE

This section provides guidance for obtaining permits, selecting parasitoid-release sites, collecting data on site characteristics, and releasing the parasitoids. For the EAB Biocontrol Program to monitor and evaluate the establishment of EAB parasitoids and the impact of EAB biocontrol, researchers and state cooperators receiving parasitoids from USDA APHIS Biological Control Production Facility must agree to submit their data to a centrally managed, online, searchable database (www.mapbiocontrol.org). The database will store data on where, when, how, under what conditions, and how many parasitoids were released and established. Personnel can use a hand-held computer (such as a Getac or Ashtech) with a built-in GPS unit to collect data in the field, or data can be entered online. The data in the GPS device should be synchronized with a central database for storage and analysis after every data collection occurrence.

OUTLINE OF PROCEDURES FOR EAB BIOCONTROL RELEASES

- **Obtain APHIS release permit.** Permits need to specify receipt of the three species of parasitoids, EAB eggs and juvenile stages, and ash host material.

- **Select a release site** in an area with good access, high density of ash trees of various sizes, and low to moderate EAB density.

- **Obtain Local Land-Use Permits**

- **Enter Data** about the site location into mapBioControl (www.mapbiocontrol.org). Take site coordinates in the center of the plot where the releases will occur. When you login, you will be asked to agree to enter release and recovery data into mapbiocontrol and agree to sample the release sites to determine which species of parasitoids have established.

- **Collect General Site Details and Physical Characteristics** using a handheld GPS unit (we recommend the Getac or Ashtech brands) or enter the information into the mapBioControl web site (www.mapbiocontrol.org).
• **Synchronize your GPS unit.** The units should be synchronized every time data are collected to prevent the loss of data.

• **Request Parasitoids for Release** once your site has been approved.

• **Collect Data on Release Trees** (size, EAB density, tree health) using a handheld GPS unit or enter data online.

• **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 using the handheld GPS device or online at mapbiocontrol.org.

• **Assess Parasitoid Establishment:** Determine if the three parasitoids are established at each site **at least one year following the final release** (Year 3). This can be done during the winter or early spring (for bark sampling or log debarking) or summer (for yellow pan traps or sentinel logs). Several methods are available for parasitoid recovery, with the choice of method depending on the specific circumstances of each release site.

**OBTAINING PERMITS**

An APHIS PPQ permit is required to release the three EAB parasitoids, *Oobius agrili*, *Spathius agrili*, and *Tetrastichus planipennisi*, in each state. To apply for the permit, complete PPQ Form 526 “Application for Permit to Move Live Plant Pests, Noxious Weeds, or Soil” online at the APHIS ePermit system: [http://www.aphis.usda.gov/permits/learn_epermits.shtml](http://www.aphis.usda.gov/permits/learn_epermits.shtml); however, you must first receive a USDA eAuthentication account: [http://www.aphis.usda.gov/permits/eauth_epermits.shtml](http://www.aphis.usda.gov/permits/eauth_epermits.shtml). The ePermit system allows you to submit and track permit applications, receive permits, and apply for renewals and amendments online. The permitting process may take four to six months to complete. Parasitoids may either be shipped as adults or as immatures inside EAB eggs or small ash logs. The ash logs will mostly contain parasitized EAB, but a few EAB could escape parasitism and emerge as adults. Therefore, your permit application should
include the host plant (ash), juvenile stages of EAB (eggs, larvae, and pupae), and each parasitoid species.

**RELEASE SITE SELECTION**

Although improved rearing methods and increased staff have allowed for the production and release of greater parasitoid numbers than in the past, each parasitoid is still rather costly to produce. Therefore, parasitoids should be released at sites where they have the highest probability of establishment. The information below will help researchers and the Rearing Facility Manager determine which sites are most appropriate for release.

**General Site Characteristics**

Locate parasitoid-release sites in naturally forested areas, woodlots, or wooded wetlands and riparian zones. To allow for parasitoid establishment and dispersal, do not select release sites that may be harvested or developed in the next 5 years. State, county, city, and township parks, recreation areas, and game areas are less likely to be disturbed than private lands. Avoid sites with excessive human activity, as well as sites along roads, trails, or railroad tracks, and in picnic areas, golf courses, and open park lands. Ash trees in such public areas may be treated with insecticide or removed.

**Minimum Acreage**

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

**Relative Density of Ash**

At least 25% of the trees over 4 cm DBH should be ash, with a higher percentage of ash even better. The percentage of ash can be estimated as <25%, 26-50%, 51-75%, or 76-100%.

**Ash Tree Size Class**

Ideally, parasitoid-release sites should contain a variety of ash size classes ranging from seedlings to mature trees. Older and highly stressed ash trees in a stand are generally attacked first by EAB and tend to die off more quickly. Although these trees are unlikely to benefit from EAB biological control, they will provide a high density of EAB eggs and larvae, increasing the probability of parasitoid reproduction at the site. Smaller trees, saplings, and seedlings provide potential for regeneration of ash trees, and will support EAB and their natural enemies following the loss of larger ash trees in the stand. *Tetrastichus*, which has a short ovipositor, appears most likely to establish in areas with some smaller, thin-barked ash trees, where EAB larvae are more accessible.

**Density of EAB**

Low to moderate EAB-population densities are recommended for potential parasitoid-release sites. Stands with many dead and dying trees are not appropriate as release sites because ash and EAB may decline before the parasitoids become well established. The most accurate method of estimating EAB density requires felling and peeling the bark from ash trees to count EAB present under the bark and along the trunk. This direct estimate of EAB density,
however, is difficult, labor intensive, destructive, and counter-productive in areas where EAB density is low. Therefore, we recommend using an indirect EAB-density estimate based on the signs and symptoms of EAB infestation in ash trees.

During the winter, before spring leaf flush, the symptoms of EAB attack on declining ash trunks include woodpecker feeding and bark scaling, bark splits, EAB-emergence holes, epicormic shoots and stump sprouts. Symptoms of dead ash trees include bark that is falling off trees, leaving exposed galleries and D-shaped exit holes (Appendix A).

After leaf flush, the condition of ash trees can be visually ranked according to the five crown-condition classes illustrating typical EAB-induced decline; crown condition 1 is a healthy canopy, 2, 3, and 4 show increasing decline and 5 is dead (Appendix C). Overall, ash trees at a potential release site should be fairly healthy, with an average crown condition of 1 to 2 (healthy or mostly healthy) and only a few trees in condition classes of 4 to 5 (dying or dead). The presence of EAB must be confirmed at each potential release site. This is done by selecting ash trees with signs of stress from a possible EAB infestation. On these potentially infested trees, remove sections of bark using a chisel or draw knife to confirm the presence of EAB galleries or EAB life stages (Appendix A). When the density of EAB is low to moderate, most EAB will be higher on the trunks, thus confirmation may require felling and debarking ash trees in the stand.

Access and local use permits

Select release sites at locations that are relatively easy to access because personnel will need to visit the site periodically for parasitoid release and recovery activities. Obtain permission from land owners for use of the site to both release parasitoids and fell some ash trees over a
period of three to four years. Keep in mind that it may take months to obtain permission or land-use permits from land owners or park managers.

**PRE-RELEASE SITE ASSESSMENT**

Prior to requesting parasitoids for release, we recommend collecting some preliminary data on site characteristics that will help the Biocontrol Rearing Facility Staff assess whether your site is appropriate for parasitoid release. We recommend collecting the data while in the field using handheld GPS units (such as Getacs or Ashtechs), which will capture the longitude and latitude data and have drop down menus for other necessary data. General Site Details and Physical Details data can also be entered online at [www.mapbiocontrol.org](http://www.mapbiocontrol.org). Be sure that when data are collected using a GPS device that you are at the location where parasitoids will be released. Do not collect GPS coordinates next to the road. Ideally the parasitoids should be released in the center of the forest, or at least 100 m from the road or other non-forested areas. Once the data are collected and the GPS unit is synchronized, the Rearing Facility Manager can review the site and determine if the site is appropriate for release. The information provided, including location, size (number of acres or hectares), percentage ash, and EAB density will assist the Rearing Facility Manager and state cooperators in prioritizing and selecting the best site(s) for parasitoid release.

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

- **Status**: Select “Proposed” because the site has not yet been approved.
- **State**
- **Date**
• Site Name

• Site Location (Enter general information such as county, town, park name, address, etc.)

• Latitude (dd.dddddd)

• Longitude (dd.dddddd)

• Plot (whether it is a release or control plot)

• Type (program or research)

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

**General Details**

• Size of wooded area in acres (you can use the measurement tools with Google Earth or ArcGIS Explorer)

• % ash (estimate)

• Dominant Tree Species

• 2nd most Dominant Tree Species (if applicable)

• 3rd most Dominant Tree Species (if applicable)

• EAB Density (Low, Medium, High)
Physical Details

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

PRE-RELEASE SITE PREPARATION

*Location of release trees*

After a site is chosen for parasitoid release, select a group of EAB-infested ash trees located near the center of your site (Fig. 1). This will be the parasitoid-release epicenter. Positive signs of EAB include woodpecker scaling and feeding, bark splits, epicormic shoots, poor crown condition, and/or EAB- emergence holes. Select one tree (it does not have to be an ash) as the release epicenter (Fig. 1, red dot in center of plot).
Mark the epicenter tree with flagging or tree paint and take the GPS coordinates.

**Plot design and tree selection**

Generally, each plot will be 4 ha (200 m x 200m) divided into sixty-four 50 m x 50 m cells surrounding the epicenter point (Fig. 1). In most plots, sampling will occur in the 16 central grid cells, however, given that not all field sites will be square (along rivers, for instance they will be long and thin) the sampling grid contains 64 cells, 16 of which will be used for sampling. You will not have to lay the grids out in the woods. Once the GPS points have been taken for the release trees, the grid cells will be laid over the points and you will be able to see where you are in relation to the grid cells when you use the GPS device.

Select three ash trees >4 cm DBH in each of the four central cells that are closest to the epicenter point; these will be the 12 parasitoid-release trees (Fig. 1, green dots in central four grid cells). Tag each tree by nailing a durable, pre-numbered, aluminum tree tag with an aluminum nail to the tree above 1 m and in consistent manner to assist those attempting to find those trees when returning to the site (NOTE: Nails will have to be removed from the tree after the project is completed if the trees are not felled and sampled). Let the nail head protrude from the tree to allow for tree growth. Also, flag all trees with brightly colored flagging so they are easier to find, and record the location of each tree using high resolution GPS.

**COLLECTION OF DATA FROM RELEASE TREES**

Collect data for each of the 12 release trees using a handheld GPS unit or enter the data on the mapBioControl web site. Collect the following information:
**Date.** Month, day, year when data were collected.

**GPS coordinates.** The GPS units in the Getac or Ashtech devices are accurate to within approximately 1-3 m. Stand as close as possible to the release tree to record the latitude and longitude in decimal degree format.

**DBH.** Record the diameter of each tree (in centimeters) at breast height (1.37 m above the forest floor).

**Crown Class.** The health of ash trees is estimated by recording the crown class on a scale of 1-5 (Appendix C). A crown class of 1 indicates a full healthy crown, while a dead tree receives a rating of 5.

**Epicormic shoots.** Epicormic shoots are sprouts that emerge from dormant buds along the trunk or branch of a tree (Appendix A). They are produced by the tree as a means of compensating for the loss of leaf surface, in this case due to damage from the EAB. Epicormic shoots tend to be green rather than brown in color and emerge directly from the trunk rather than following the normal branching pattern of the tree. Count or estimate the number of epicormic shoots on the upper and lower half of the main trunk of each tree.

**Number of EAB exit holes.** Count the number of EAB exit holes (Appendix A) you can detect on the trunk up to approximately 1.5 m.

**Number of bark splits.** Ash bark often will split when there is an EAB gallery beneath the bark (Appendix A). Record the number of bark splits visible on the lower 1.5 m of trunk.

**Woodpecker feeding damage.** Woodpeckers feed on EAB larvae located under the bark and leave a characteristic hole in the bark. In addition, foraging woodpeckers often remove
flakes of bark, leaving light colored patches on the trunk (Appendix A). Record whether woodpecker feeding damage is evident in the upper and lower half of the tree.

PARASITOID RELEASE

WHICH SPECIES TO RELEASE

Prior to 2012, the EAB Biocontrol Program provided the three EAB biocontrol agents to each state for release upon request. Releases of Tetrastichus and Oobius will continue in all states. Spathius agrili, however, may not be surviving adequately in northern regions. Therefore, the EAB Biological Control Program will now provide S. agrili for programmatic releases in areas below the 40th parallel north. If any portion of a county falls on or below the 40th parallel north, that county is eligible for S. agrili releases. Release and evaluation efforts in more southern areas, particularly in Kentucky, Missouri, Tennessee, Virginia and West Virginia have been more recent and performance of S. agrili is not yet known. Monitoring for S. agrili establishment will continue at sites where S. agrili was released in the past and at new sites south of the 40th parallel.

TIMING OF RELEASE

Spring Tetrastichus and Spathius releases: In more northernly states or locations early in the EAB-infestation cycle when EAB densities are low, EAB may take two years to develop to the adult stage. Eggs laid late in the year also may fail to develop to the J-larval stage. Under these circumstances, the third- and fourth-instar larvae needed for parasitism by S. agrili and T. planipennisi are present during spring, summer, and fall. After 300 growing
degree days (GDD base 50ºF) have accumulated in your area, we will contact you about shipping the larval parasitoids, *Tetrastichus* and *Spathius*, for a spring release.

**Summer Oobius releases:** *Oobius agrili* lays its eggs inside EAB eggs, therefore, releases should be initiated 2-3 weeks after EAB adults begin to emerge (EAB adults take several weeks to mature before mating and laying eggs). We will contact you about receiving shipments of *Oobius* when ~800 GDD have accumulated. In northern areas or areas with late emergence of EAB adults, *Oobius* releases may continue through August. Two helpful handouts on the timing of EAB emergence are online at:


**Late summer/Fall release of Tetrastichus and Spathius:** Both larval parasitoids attack mature (late 3rd and 4th instar) EAB larvae. In southern areas and late in the EAB-infestation cycle when population densities are high, EAB populations are more synchronized and mature larvae are more abundant from mid-summer to fall. Begin late summer/fall releases when 1800 GDD have accumulated. Because of diapause considerations, we find that *Spathius agrili* should not be released after the end of August, however *Tetrastichus* can overwinter in any stage and releases can continue as long as field conditions are favorable for adult oviposition and the EAB larvae haven’t entered the overwintering chambers.

**NUMBER AND FREQUENCY OF RELEASES**

The minimum numbers of parasitoids recommended for release are listed below by species. The actual numbers shipped, however, will vary depending on total availability during any given week and the number of release sites requiring insects. Whenever more parasitoids are
available they will be shipped and should be released because the probability of
establishment is increased when more parasitoids are released. Each release will consist of a
specified number of female parasitoids previously exposed to male parasitoids for mating
and an unspecified number of males. Because weather patterns in any given year can impact
the synchrony between availability of the appropriate stages of EAB and release timing,
releases should be made during two years.

**Spring Tetrastichus and Spathius release:** You will be shipped a minimum of 200
*Tetrastichus* and 200 *Spathius* females (where appropriate) every other week for 5 weeks
beginning when 300 GDD have accumulated, for a minimum of 600 females released for
each species. When more than 200 parasitoids are available for shipment, you will receive
more for release.

**Oobius release:** Release a minimum of 100 *Oobius* per week for four to six weeks for a total
of a minimum of 400 to 600 released.

**Late summer Tetrastichus and Spathius release:** Release a minimum of 200 females of
each species every other week for 5 weeks beginning when 1800 GDD have accumulated. A
minimum of 600 females will be released for each species.

**REQUESTING PARASITOIDS**

Email all parasitoid requests to Stephanie Likens (Stephanie.Likens@aphis.usda.gov) and
copy Jonathan Lelito (Jonathan.Lelito@aphis.usda.gov). The Request Tab on
mapbiocontrol.org is currently under development and should not be used at this time.
RECEIPT OF PARASITOIDS

Parasitoids are shipped by overnight delivery in a cooler, and should arrive by 10:30 AM at most locations. Adult *Spathius and Tetrastichus* will be shipped either as developing pupae inside ash bolts or as adults in 16-oz plastic cups with screening on the lid. Ash bolts will contain a hanging device for easy mounting on release trees. Honey will be smeared on the screening as a source of food for the adult parasitoids in cups. Adult *Oobius* will be shipped in plastic cups. Honey will be streaked on the side of the cup. *Oobius* may also be provided as pupae inside EAB eggs on paper inside plastic cups that can be mounted on release trees.

*The parasitoids should be released the same day they are received.* If you are unable to release parasitoids on the scheduled day because of personnel shortages or adverse weather conditions are predicted, contact the Biological Control Release Coordinator at least a day in advance to arrange for a different shipping date. After arrival, transport the parasitoids in the cooler to the release site and release on the designated trees.

CARE OF ADULT PARASITOIDS IF RELEASE IS DELAYED

If there is an unforeseen delay caused by late delivery or unexpected weather conditions, the parasitoids will require your care to survive in their shipment cups beyond the day of arrival. To care for the parasitoids, unseal and open the cooler, remove and open the bags. Inside each bag will be a number of labeled cups or vials containing small groups of live parasitoids. To maintain sufficient ambient moisture for the parasitoids, we recommend placing the rearing cups in a clear plastic storage tub with moistened paper toweling. Before placing the cups in the plastic tub, check each cup for the presence of honey. Honey provides the parasitoids with food and some moisture during shipping. The *Oobius* should
not require additional honey and easily escape or are injured when the cup is opened. If no 
honey is visible on the screening on the lids of the cups with *Spathius* or *Tetrastichus*, put 
two or three drops of honey on the screening and gently smear it.

**TRANSPORTING PARASITOIDS TO FIELD SITES**

Carry the cups or infested logs inside the cooler 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 cooler out of direct sunlight or other potentially hot (e.g., a 
sealed vehicle) environments. The trunk of a vehicle will suffice, but an air conditioned 
interior is even better, provided the vehicle will not be allowed to sit unattended in the sun 
for any period. *Keep the cooler in the shade at all times* because parasitoids are extremely 
sensitive to overheating. Keep the cooler closed except to remove the cups or logs with 
parasitoids for release. Carry the cooler carefully and avoid sudden movements. Parasitoids 
are extremely small and susceptible to drowning in droplets of water or honey if the cup is 
 inadvertently shocked or dropped.

**RELEASE OF PARASITOIDS**

*Adult Parasitoids.* If possible, release the parasitoids in the morning or evening so they can 
move about in the environment before the onset of high afternoon temperatures. Parasitoids 
should be released onto the 12 pre-designated tagged release trees. Carefully remove the lid 
and place the cup or vial next to the trunk of the tree. If *Oobius* are resting in the crack 
between the cup and the lid, open the lid on the side nearest them to avoid crushing them. 
On warm sunny days, most of the parasitoids will crawl up to the lip of the cup or vial 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 it against the tree, causing the parasitoids to jump or fly onto the tree trunk. Move the cups from tree to tree to ensure the number of each species is somewhat evenly distributed across the release trees.

**Parasitoid Pupae in Logs.** The small ash logs containing parasitoid pupae will come with a pre-drilled hole, through which you can insert a long nail (4-5”) to nail into the tree trunk or hang from a branch using a zip-tie or sturdy twine. You will need some large nails with wide heads and a hammer to hang the logs or zip-ties to secure the logs to the branches of smaller trees. Other options for hanging the small logs include tapping a nail into the top of the bolt and hanging the bolt from a short piece of twine, wrapped around a horizontal branch of the tree – this is particularly effective on large trees where trunk diameter interferes with the hanging of the log on the side of the trunk. The logs should remain in the field for at least 3-4 weeks to assure that all the parasitoids have emerged as adults. Remove the nails from the trees when you recover the logs because nails will harm sawmill equipment if the trees are harvested.

Every time you release parasitoids, enter the following information into your GPS device or mapbiocontrol:

- **Release Date**
- **Release time**
- **Weather Conditions (Sunny, Partly Cloudy, Foggy, Light Rain, Moderate Rain, Heavy Rain, Thunderstorms)**
- **Wind Speed (Light, Moderate, Strong)**
EVALUATING PARASITOID ESTABLISHMENT

Several methods have been developed that can successfully recover the three exotic parasitoids of EAB. Unfortunately none of the methods is consistently more effective than the others, and there are circumstances where parasitoids are recovered using one method but not others. The method we recommend cooperators use as a first choice is felling trees and either debarking them or putting the logs in emergence tubes. Using these methods we are certain that recovered parasitoids attacked EAB (tree debarking) or at least attacked an insect (probably EAB) in an ash log (logs in emergence tubes). If samples are taken during the winter, these methods can recover all three parasitoid species. However, there will be situations where cutting trees is impractical or not allowed, and we present alternative methods including yellow pan traps, egg- or larval-sentinel logs, and searching for EAB eggs. All sampling to recover parasitoids should begin at least one year after the final release at a given site. By waiting at least one year, parasitoid populations will have time to build up in and around the release site, increasing the probability of detecting the parasitoids using current methods. Below we describe the three parasitoid species and how their life cycle affects recovery sampling:
*Tetrastichus planipennisi* is a gregarious endoparasitoid (internal parasitoid) of EAB larvae, and 20 to >100 *Tetrastichus* larvae develop inside their host. *Tetrastichus* may have three to four generations per year. An EAB larva parasitized by *Tetrastichus* may 1) look healthy; 2) appear lumpy like a “braided rope”; 3) be replaced by a mass of small grub-like larvae (white), pupae (color ranges white to bluish-black) and/or adults (dark metallic blue); or, 4) be consumed, leaving only the head and tail of the EAB larva and small black spots in the gallery (the spots are waste excreted by each *Tetrastichus* adult after pupation is complete) (Appendix B). The parasitoids spend the winter in the EAB gallery and may be recovered by debarking ash trees. The insects will also emerge from logs brought into a warm environment and can be captured by placing the logs in cardboard tubes fitted with collection jars. *Tetrastichus* will attack larvae in sentinel logs, and adults have been recovered in yellow pan traps in the late summer.

*Spathius agrili* is a gregarious ectoparasitoid (external parasitoid) of EAB larvae, and all life stages live on the outside of the host. *Spathius* eggs and small larvae are difficult to see with the naked eye, but by late fall, most will be large larvae (Appendix B) or will have spun silken cocoons and will be fairly easy to see in the EAB galleries (Appendix B). *Spathius* requires a period of chill to break diapause, thus cutting trees for debarking or rearing in cardboard tubes should not take place before January. Like *Tetrastichus*, this larval parasitoid can sometimes be recovered in sentinel logs and yellow pan traps.

*Oobius agrili* spends the winter in diapause inside EAB eggs, which are difficult, but not impossible, to find sheltered between layers of bark and in bark crevices. EAB eggs are light brown or gold, whereas *Oobius*-parasitized eggs are often dark brown or black in color (Appendix B). One can collect EAB eggs directly from the bark of ash trees, placing the
eggs in a warm environment until adult parasitoids emerge. Alternatively, pieces of bark containing parasitized eggs can be collected and placed in cardboard emergence tubes in a warm environment for parasitoid emergence. Small ash logs on which EAB females have deposited eggs in the laboratory can be brought to the field as egg sentinel logs to recover Oobius, but they take some time and expertise to construct. To date, Oobius have not been recovered in yellow pan traps.

NOTE: If you would like examples of parasitoid adults, larvae, pupae or cocoons to help with field identification, please contact the Rearing Facility Manager for specimens. If you have questions about where to purchase supplies and/or questions about how to construct sentinel logs, yellow pan traps, or emergence tubes, please call one of the authors.

TREE FELLING AND DEBARKING OR EMERGING IN CARDBOARD TUBES

Cutting trees to determine parasitoid establishment should be done from January through April (after the insects have received enough cold to break diapause), at least one year after the final release at a given site. Select four trees near the release epicenter that are alive (based on bark peeling and confirmation of live phloem), show signs of damage due to EAB, and are not too large to cut down safely. Select and fell living EAB-infested trees that are less than 10-inches DBH. Record the tree number, GPS coordinate, date the tree was felled and the DBH of each tree. If you have a handheld GPS unit, you can enter these data in the field before felling the tree.
OPTION 1: DEBARKING

Before debarking the logs to recover larval parasitoids, search for parasitized EAB eggs on the bark or put samples of the bark in rearing tubes to collect emerging *Oobius* adults.

**Placing Bark in Rearing Tubes to Recover**

*Oobius.* For each 1 m length of tree, carefully remove 40 cm$^2$ of bark (approximately 10 X 40 cm or 20 X 20 cm depending on the size of the log). Place the pieces of bark in a paper bag (plastic will cause the bark to mold) for storage or directly in emergence tubes and hold in well-lit area at room temperature. Paper bags containing bark samples can be refrigerated (4°C) in a large plastic bag for up to three months.

What will I need to make rearing tubes to rear *Oobius* from Bark Samples?

1. a well-lit room, heated at 70 to 80°F, with shelving to hold the rearing tubes
2. 4-inch diameter cardboard tubes for different diameter logs; number of rearing tubes depends on the amount of space and shelving
3. *Oobius*-rearing tubes are cut down to 10-inches in length
4. recessed end-caps that fit inside the end of each tube
5. plastic-adhering black spray paint
6. small plastic funnels
7. large refrigerator or cold-room at 34°C
8. urine specimen cups
9. hole cutter slightly smaller than the diameter of the specimen cup lid
10. hole cutter smaller than the diameter of widest part of funnel
11. hot glue gun and glue sticks
12. fine forceps
13. 1 mL screw-top plastic vials with o-rings
14. Magnifying glasses or dissecting microscope

The following is a brief description of how to make and start using rearing tubes for emergence of *Oobius*: Purchase 4-inch diameter cardboard mailing tubes with caps for both ends. Cut the tubes down to 10-inch lengths.

Spray paint the cardboard-tube caps and funnels black with plastic-adhering spray paint. At the base of each funnel, cut off the stem. Modify the cap at the front of the tube (toward light) by cutting a hole (use a hole-cutter), slightly smaller than the diameter of a specimen-cup lid, in the center of the cap. Modify lids of specimen cups by cutting a hole (use a hole-cutter) large enough to allow the funnel be seated flush with the top of the lid. Hot-glue the funnel into the hole in the lid with the funnel facing inward. Hot-glue the lid to the specimen cup into the hole in the cardboard-tube cap with the threaded side facing out. Screw the collection jar onto its lid. *Oobius* will be attracted to the small hole of light in the specimen cup, which should be checked every two days for emerged parasitoids; remove and cap the emergence cup and freeze it. After a day or two and the parasitoids are dead, place parasitoids into 1.5 mL microcentrifuge tubes using fine-forceps using a dissecting microscope (due to small size of *Oobius*). Using a fine-tipped pen such as a Sharpie®, label each sample with the state, site, date, and tree number on each small tube. Keep adults
stored in the freezer until the samples are sent to the APHIS Biocontrol Rearing Facility for identification. Enter the date and number of specimens shipped into MapBioControl.

To further evaluate these bark samples for egg parasitism by *Oobius*, the dried bark can be sifted through plastic window screening. The EAB eggs are then readily sorted from the sifted debris in a white porcelain baking dish under a dissecting microscope or magnifying glasses. For a detailed analyses of parasitism among egg samples, place the eggs in a small petri dish or vial and send to Leah Bauer, USDA Forest Service, Northern Research Station, 1407 S. Harrison Rd., East Lansing, MI 48823.

**Peeling Logs to Recover Larval Parasitoids:**

Both species of larval parasitoid can be found in EAB galleries under the bark. Logs are easiest to peel if debarked soon after felling, but if you need to store the ash logs in a cold chamber, seal the ends (for example Anchorseal®) to reduce moisture loss. If the bark is thick, scrape the outer bark off with a draw knife and carefully remove the phloem with a chisel. Phloem will easily separate from the outer sapwood when the ash logs are fairly fresh. Inspect all EAB galleries for signs of parasitized larvae (see Appendix B for photos of parasitized EAB). Carefully remove the EAB larva along with the parasitoid larvae or cocoons and place them in a small Petri Dish with a tight fitting lid (Fisher Scientific 50 X 9mm dishes – catalog number 08-757-105 is a good choice). Using a fine-tipped Sharpie, label each Petri Dish with the state, site, tree number, and date. Mail the specimens within one week to the Rearing Facility Manager for identification. For each tree, record the number of live EAB larvae, solitary larvae or
cocoons (probably *Atanycolus* – just count, do not ship), gregarious larvae (ship these for identification) and gregarious cocoons (ship these for identification). Enter these data into MapBioControl.

**Emerging Parasitoids from Logs in Rearing Tubes:** Instead of debarking, the logs can be placed in large cardboard tubes known as “rearing tubes”, which are held on shelves in a well-lit room at ~70 to 80°F for at least three months. *Oobius agrili*, *T. planipennisi*, *S. agrili*, and most other insects in and on the logs will emerge within one month. However, some *S. agrili* take more than three months to break diapause. Store the logs in a cold room or refrigerator (~34°F) prior to placement in the rearing tubes; ash logs can be stored for about six months. The number of rearing tubes needed depends on many factors including size of the room, the number and size of the tubes and logs, and length of time the logs are held in the tubes. Generally, the logs are held for about six weeks with one large or several smaller logs fit inside a single tube. Rearing parasitoids from logs takes up considerable space and attention to detail, it may be more practical than debarking the logs and rearing out the parasitoids.

**What will I need to make rearing tubes for parasitoid emergence?**

1. a well-lit room, heated at 70 to 80°F, with shelving to hold the rearing tubes
2. 8-, 10-, and 12-inch diameter cardboard tubes for different diameter logs; number of rearing tubes depends on the amount of space and shelving

3. rearing tubes are typically cut down to 28- to 30-inches in length

4. recessed end-caps that fit inside the end of each tube

5. plastic-adhering black spray paint

6. small plastic funnels

7. large refrigerator or cold-room at 34°C

8. urine specimen cups

9. hole cutter slightly smaller than the diameter of the specimen cup lid

10. hot glue gun and glue sticks

11. Forceps

12. 1 mL screw-top plastic vials with o-rings

13. Magnifying glasses or dissecting microscope

The following is a brief description of how to make and start using rearing tubes for parasitoid emergence: cardboard tubes are sold in various diameters and lengths. Cut them down to a convenient length based on the size of the room, considering the weight of fresh ash logs, etc. Spray paint the end-caps black and snap one into the back of the tube. Modify the front end-cap by cutting a hole with a hole-cutter slightly less than the diameter of the lid of the urine specimen cups. Modify the lids of each specimen cup by cutting a hole (use a hole-cutter) large enough to allow the funnel be seated flush with the top of the lid. Modify the funnel by using a single-edged razor blade to notch
the funnel stem 1/2" from the tip and then to slit the end of the stem lengthwise down to the notch removing one side to create an EAB “launch pad”.

Hot-glue the funnel into the hole in the lid with the funnel facing inward. With the threads facing out, hot-glue the lid of the cup onto the cap of the tube. The cup can then be screwed onto the outside of the cap, and the cap can be snapped into the end of the rearing tube after the log is inserted. Seal the ends of each log before placing them in the tubes using either a paintable sealant or dipped into melted paraffin. After log(s) are inside the tubes, place narrow shims under and between logs; this provides space for the parasitoids to emerge.

Every day or two, collect the emerged insects by unscrewing the cup, screwing on another lid quickly to avoid loss, and replacing the cup on the tube with an empty one. Freeze the insects and carefully transfer the insects to the small vials and label each vial with the state, site, date, and tree number. Keep the vials frozen until shipped overnight to the Rearing Facility Manager for identification. Enter the recovery data into MapBioControl.

YELLOW PAN TRAPS

Many adult bee and wasp species are attracted to the color yellow. In Michigan field sites where the three introduced emerald ash borer (EAB) parasitoids are established, we found yellow pan traps (YPTs) were effective at trapping the two larval parasitoids *Tetrastichus planipennisi* and *Spathius agrili*. YPTs did capture the EAB egg parasitoid *Oobius agrili* at some sites in MI, but further testing is required before we know if this is an efficient method for recovering *Oobius*. Other known EAB larval parasitoids (e.g. *Atanycolus*, *Spathius*, *Phasgonophora sulcata*, *Balcha indica*) were also trapped, along with many other species of bees, wasps, flies, hemipterans and beetles. YPTs are simple and inexpensive to make.
What will I need to make one YPT?

1. two 12-oz yellow plastic bowls (color: yellow sunshine; manufacturer: Festive Occasion, East Providence, RI 01916)
2. one 6-inch right-angled shelf-bracket
3. three 1.25-inch long wood screws
4. weather-proof marking pen (e.g. Sharpie) and grease pencil (needed if bowls are wet)
5. three 6-inch zip-ties
6. 20% solution of clear (not pink or green) propylene glycol (non-toxic antifreeze) diluted with water. You can type “food grade propylene glycol” into a search engine to find a supplier.
7. rechargeable portable electric screw-driver with bit and extra battery pack
8. unscented dish detergent

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

1. One paint filter per pan trap per sample occasion
2. One Ziploc bag per pan trap per sample occasion
3. Permanent markers
4. Pencil and paper

How are the YPTs mounted? Using the electric screw-driver, attach a shelf-bracket to the trunk of a living ash tree infested with EAB. Attach the bracket ~5 feet above the ground with the three wood screws.
What about those two yellow bowls? One yellow bowl is used as a “holding-bowl.” It is attached to the shelf-bracket with zip-ties threaded through the three shelf-bracket holes (on the horizontal surface). The zip ties should be threaded through pairs of holes punched into the holding bowl with a paper punch (0.5 to 1.0 cm below the lip) and then through the hole in the shelf bracket. There are two holes in the shelf bracket next to the tree and one hole at the tip. Do not pull zip-ties too tightly to avoid distorting the holding-bowl. To provide drainage in the holding-bowl, cut a hole (~1-inch-square) in the bottom with a utility knife.

The second yellow bowl or “trapping-bowl” will hold the liquid that traps insects. It rests inside the holding-bowl. To prevent overflow from the trapping-bowl after rainfall, punch at least 6 drainage holes just below the lip. Hot-glue a strip of fine-mesh screening (e.g. organdy) over the drainage holes to prevent loss of specimens during overflow. After the bracket and holding-bowl are mounted on the tree, set the trapping-bowl in the holding-bowl. Fill the trapping-bowl ~¾-full with the 20% propylene glycol solution (make sure that the propylene glycol is clear, not pink). Add one drop of unscented dish detergent to break the surface tension of the solution. This will allow inquisitive insects to become entrapped in the liquid. You will need to empty the trapping-bowl after three to seven days to avoid possible loss of the sample due to weather, vandals, wildlife, decay, etc. We find that it is most convenient to collect samples once per week; adding fresh propylene glycol after collecting the first sample and continuing weekly samples.
**How many YPTs should I deploy and where?** Deploy a total of 15 YPTs with one YPT per ash tree at your EAB biocontrol release site. If possible, select an ash tree at least 4-inch DBH showing some symptoms of EAB infestation (e.g. wood-pecker feeding, epicormic shoots) with crown class 2, 3, or 4. Do not put the traps on dead ash trees.

Locate the traps in the four 50-m x 50-m grid-cells surrounding the parasitoid-release epicenter. Assuming you can find the appropriate trees, place three pan traps in each of the central four cells (Fig. 1). If the site is long and thin rather than square, then you may have to select four cells in a row rather than in a square. And if most of the trees in the center are dead, you may need to move outside the central four cells to find sufficient live trees onto which to hang the traps.

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

**When should the YPTs be deployed?** Deploy YPTs at EAB biocontrol release sites the year following the final parasitoid releases. The adult parasitoids fly throughout the spring, summer, and early fall. However, populations build throughout the summer. We have recovered *Oobius* in YPT’s at a few sites, and if you want to trap for *Oobius*, deploy YPT’s in July. For larval parasitoids, the last three weeks of August and the first week of September are the best times for sampling.
**How long do I leave YPTs in the field?** The YPTs can be left on the trees for three to seven 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 a heavy downpour, however, you might want to consider collecting the samples early.

**How is the insect sample collected from the YPT?** After locating the YPT in the field, pour the contents of the trap (insects plus liquid) through a paint filter. The propylene glycol is not toxic and can be poured on the ground. Fold the paint filter and place each one separately into a labeled Ziploc bag or whirl pak. Use a permanent marker for the label (include state, site, YPT-ID number, and date). Store samples in the refrigerator and ship within one week. If shipment is delayed, store the samples in the freezer.

**What do I do with these samples?** Send the samples by overnight shipping to Juli Gould, 1398 West Truck Road, Buzzards Bay, MA  02542. Enter recovery data into MapBiocontrol.

**SENTINEL LOGS**

Sentinel Logs are small ash logs containing EAB eggs or larvae that are placed in the field to attract female parasitoids. They are especially useful when EAB density is low and finding naturally occurring EAB eggs or larvae is difficult or time consuming.
**Egg Sentinel Logs:** Egg-sentinel logs (ESLs) are small ash logs on which EAB adults have laid eggs in the laboratory. Plan to deploy ESLs on or near the original *Oobius*-release trees for about 10 days (max. 14 days) between 1800 and 2500 GDD50 (growing degree days with a base of 50) (early to mid-August in central Lower Michigan). ESLs with an average of ~100 eggs/log can be produced by exposing freshly cut ash logs (6- to 7-cm diameter × 25-cm long) for two to three days to 20 gravid EAB females (mated females average 33-days-old; range 23 to 52 days) and 10 males in 3.8-L ventilated plastic jars with fresh ash leaves with petiole sealed in a water-filled vial. To reduce EAB morality, change the leaves and the jars daily, and remove and replace dead EABs with live ones of similar age and sex. To prepare the log for exposure to EAB, dip each end in paraffin or other log sealant, and wrap it loosely with a spiral of curling ribbon (attach each end with a thumb tack). The ribbon encourages EABs to oviposit on the log. After two to three days, remove the ribbon, count and circle the eggs with a fine-tip marker, and place the ribbon back over the eggs (use thumb tacks to support the ribbon over spiral of eggs). The ribbon reduces egg predation in the field. Ants are the most common predator of EAB eggs in urban and suburban areas and are not readily deterred by the ribbon. To limit ant predation on the ESLs, therefore, select ash trees for placement of ESLs in wooded areas as far away from pavement (e.g. sidewalks, roads, buildings, parking lots, etc.) as possible. To deploy an ESL, attach an eyelet screw to one end of the log and attach it to the tree with a nail; place another nail underneath the log and bend it up into the bottom of the log to help stabilize it. Be sure to remove the nails when you retrieve the logs from the field. After retrieving the logs from
the field, remove the EAB eggs (on a small bark flake using utility knife). Place the eggs from each ESL in 100-mm ventilated Petri dishes, tape dish closed, label with the state, site, and ESL number, and ship immediately to the Rearing Facility Manager to assess parasitism of the EAB eggs.

**Larval Sentinel Logs.** Larval-sentinel logs (LSLs) can be made by cutting small ash logs (~5 cm dia × 18 cm long), inserting five 3\textsuperscript{rd}- or 4\textsuperscript{th}-instar EAB larvae in chambers cut under bark flaps, securing the bark flaps with rubber bands, sealing the ends of the logs with Parafilm or AnchorSeal, and hanging them on ash trees. Large screw eyes should be inserted into one end of each log, and logs should be hung on release trees at your release site. Retrieve the logs after they have been in the field for 1-2 weeks. Carefully split the logs and remove each larvae, place in a 100-mm Petri dish, tape close, label, and send the larvae to the Biocontrol Rearing Facility.

**COLLECTION OF EAB EGGS**

Evidence of *Oobius agrili* parasitism can be found by collecting EAB eggs. Although this can be at any time during the year due to persistence of EAB eggs on ash bark,
*Oobius* populations tend to be highest during the fall (through November). EAB eggs are laid in bark crevices or under bark flaps, so use a utility or pocket knife to gently lift off layers of the bark to find eggs. A headband magnifier, such as an Optivisor®, or magnifying reading glasses can help the collector see the small eggs. Gently remove the egg on a thin layer of bark with the point of the knife; place the eggs from each sample in 100-cm diameter ventilated Petri dish, tape the dish closed. Label each vial with the state, site, date, and tree number. Send the samples as soon as possible to the Rearing Facility Manager for insect emergence and identification, and enter collection data (Appendix H) into Mapbiocontrol. If the samples must be held for more than two weeks, they should be stored in the refrigerator.

**ENTER RECOVERY DATA**

It is critical that the EAB Biological Control Program have data on where EAB parasitoids are establishing. Once you have completed surveys to detect established parasitoids, enter the data into [www.mapbiocontrol.org](http://www.mapbiocontrol.org). Data on samples that were collected but no parasitoids were recovered are also critical. When you enter the mapbiocontrol.org web site, click on RECOVERY in the green banner at the top. Click the New button to enter new data. You will be asked to enter the following data:

- **Trap ID:** This is the unique trap ID that you have given each yellow pan trap, sentinel log, or cut tree. This data is critical because the scientists who identify collected parasitoids need to match the identified insects to the sites and locations where the parasitoids were recovered.
- **Latitude** (dd.dddddd)
- **Longitude** (dd.dddd)
• **Site ID** Once you type in the Latitude and Longitude of your sample, the database will select some nearby sites from which to choose. Select the appropriate site. If you happened to find parasitoids not connected with any particular release or control site, simply select NO Site.

• **State**

• **Date Sample Collected**

• **Sample Method** (Yellow Pan Trap, Tree Debarking, Logs in Tubes, Bark in Tubes, Sentinel Eggs, Sentinel Larvae, Egg Collection, Other)

• **Number of Samples**

• **Possible EAB Parasitoids Recovered?** Yes or No

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

**Forest Type**

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

**Ash Health Assessment**

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

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

**EAB Life-Stages**

*EAB eggs* (newly laid egg is white, clutches of eggs that were under bark flakes, single eggs in bark crevices)

*EAB larvae* (1-4 indicates the four instars)
Appendix A – EAB Life Stages and Damage

EAB J-shaped larvae, pre-pupa, pupae
Appendix A – EAB Life Stages and Damage

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

*EAB Adult*

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A-3
Appendix A – EAB Life Stages and Damage

Signs of EAB infestation

Thinning Ash Crowns

Epicormic Shoots in Winter and Summer

J. Gould

L. Bauer

J. Gould
Appendix A – EAB Life Stages and Damage

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

Damage from Woodpecker Feeding
Appendix A – EAB Life Stages and Damage

**D-shaped exit holes**

**Larval Galleries**
Appendix A – EAB Life Stages and Damage

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

Life stages of EAB Parasitoids

*Spathius agrili*

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

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

Silken cocoons of *S. agrili* in the host gallery contain mature larvae or pupae.
Appendix B - Parasitoid Life Stages

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

*Tetrastichus planipennisi*

*Im mature T. planipennisi* larvae inside EAB 

Mature *T. planipennisi* larvae inside an EAB larva
Appendix B - Parasitoid Life Stages

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

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

*T. planipennisi* female lays eggs in an EAB larva through ash tree bark.
Appendix B - Parasitoid Life Stages

**Oobius agrili**

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

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

*O. agrili* female parasitize EAB eggs laid on ash bark.
Appendix B - Parasitoid Life Stages

D. Miller
Appendix C – Crown Condition of Ash Trees


After full leaf flush, rank the canopy or crown conditions of ash trees from 1 to 5. Crown-class 1 is a healthy tree with no obvious signs of decline, 2, 3, and 4 show successive canopy thinning, and 5 is a dead tree.
Appendix D – Helpful Links

mapBioControl (to enter release and recovery data)
www.mapbiocontrol.org

e-authentication application

e-permits

Growing Degree Days
http://www.epc.ncep.noaa.gov/products/analysis_monitoring/cdus/degree_days/grodgee.txt
http://uspest.org/US/
For MI: http://www.enviroweather.msu.edu/homeMap.php

Timing of EAB Emergence

General EAB Information

EAB Program Manual

APHIS Emerald Ash Borer Home Page

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

Forest Service EAB Information
http://na.fs.fed.us/fhp/eab/
http://nrs.fs.fed.us/disturbance/invasive_species/eab/control_management/biological_control/