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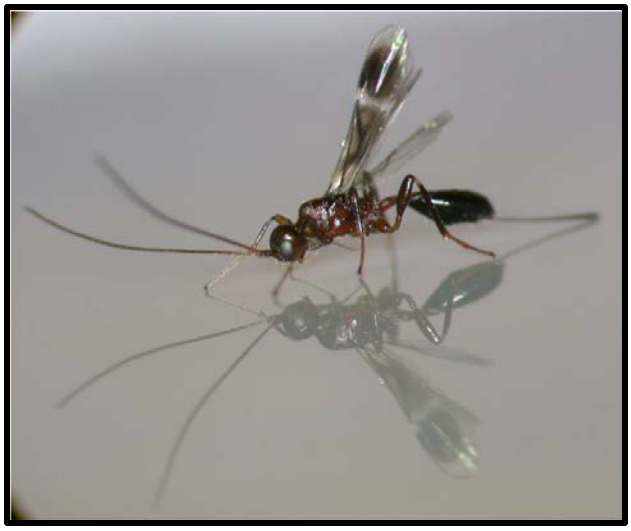
Emerald Ash Borer Biological Control Release and Recovery Guidelines



Emerald Ash Borer, *Agrilus planipennis* (Fairmaire), Biological Control Release and Recovery Guidelines – April 2012

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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 February 2011, EAB infestations were known in fifteen states (Indiana, Illinois, Kentucky, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Virginia, West Virginia, Wisconsin, Iowa, and Tennessee) 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) 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 emergence 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 and evenings. The adults of both sexes are strong fliers.

Eggs

A female EAB may lay >100 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, pre-pupae, 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. Larger larvae complete development the following spring, whereas smaller 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 the EAB galleries, and the tree shows few outward signs of infestation. On thin-barked 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 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 egg parasitoids were reared from > 6,000 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 *Agrilus* spp. and for EAB in China.

BIOLOGY OF EAB PARASITOIDS

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

Spathius agrili parasitizes up to 90% of EAB larvae in ash trees in some parts of China. Female *Spathius* parasitize EAB larvae by drilling through the bark (Appendix B) and laying an average of 8 eggs on the outside of its host. The hatching parasitoid larvae (Appendix B) feed and develop on the EAB larva, causing its death. The cycle is repeated 1-2 times each summer and fall depending on climate. *Spathius* 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.

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 *Tetrastichus* is similar to that of *Spathius,* however, the female parasitoid lays eggs inside EAB larvae where the parasitoid larvae grow, eventually killing their host. *Tetrastichus* 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 EAB-infested 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.

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 dissected from ash bolts, which are harvested at nearby field sites, however EAB larvae can also be raised on an EAB artificial diet or in ash bolts.

To increase rearing efficiency, exposure of naturally-infested ash bolts containing EAB larvae to *Spathius* or *Tetrastichus* is being evaluated for parasitoid rearing. When implemented, this technique will change how adult parasitoids are produced and released into the field; the entire ash bolt containing artificially grown hosts could be sent to a field site and emergence of the parasitoids could occur naturally under field conditions.

PROJECT STATUS

In January 2009, a Biological Control Production Facility became operational in Brighton, MI. As of February 2012, this facility has reared and released over 444,000 EAB parasitoids in twelve 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 temperatures are colder than in Tianjin, China, where *S. agrili* was collected. Host specificity testing is nearly complete for the *Spathius* from Russia.

FIELD RELEASE

This section provides guidance for obtaining permits, selecting parasitoid-release sites, collecting data on site characteristics, and releasing the parasitoids. In order for the program to monitor and evaluate 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. 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. Periodically, the data in the GPS device must be synchronized with a central database for storage and analysis.

OUTLINE OF PROCEDURES FOR EAB BIOCONTROL RELEASES

- **Obtain APHIS release permit. Permits need to specify receipt of EAB 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**
- **Complete and Submit Pre-Release Site Assessment** using a handheld GPS unit (we recommend the Getac or Ashtec brands) or the mapBioControl web site (www.mapbiocontrol.org). Take site coordinates in the center of the plot where the releases will occur.
- **Request Parasitoids for Release** once your site has been approved. When you submit the request online, you will be asked to agree to enter release data into mapbiocontrol and agree to sample the release sites to determine which species of parasitoids have established.

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

- **Synchronize your GPS unit.** The units should be synchronized every time data are collected to prevent the loss of data.

- **Collect Data on Site Characteristics** (physical attributes of the site, basal area of ash and other tree species, ash health) using the handheld GPS unit.

- **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:** During the winter or early spring **at least one year following the final release** at a site (Year 3), determine if the three parasitoids are established. 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." This form is available online (http://www.aphis.usda.gov/permits/learn_epermits.shtml) using the APHIS ePermit system; however, you must first receive a USDA eAuthentication account: 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 pupae inside small ash logs. The ash logs will mostly contain parasitized EAB, but a few EAB could escape parasitism and emerge as

adults. Your permit application should therefore include the three parasitoid species (eggs, larvae, and pupae), EAB host material, and ash material.

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, the number of release sites may exceed production capacity. 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%. Once a site is selected for release, the percentage of ash will be quantified.

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* appears most likely to establish in areas dominated by smaller, thin-barked ash trees, where they can find the most readily accessible EAB hosts.

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 high 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. 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

Complete the Biological Control Pre-release Site Assessment (described in Appendix D) once a potential site has been selected. 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. Pre-release assessment data can also be entered online at www.mapbiocontrol.org. Be sure that when you collect data using a GPS device that you are standing in the part of the plot where you will eventually release the insects. 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.

PRE-RELEASE SITE PREPARATION

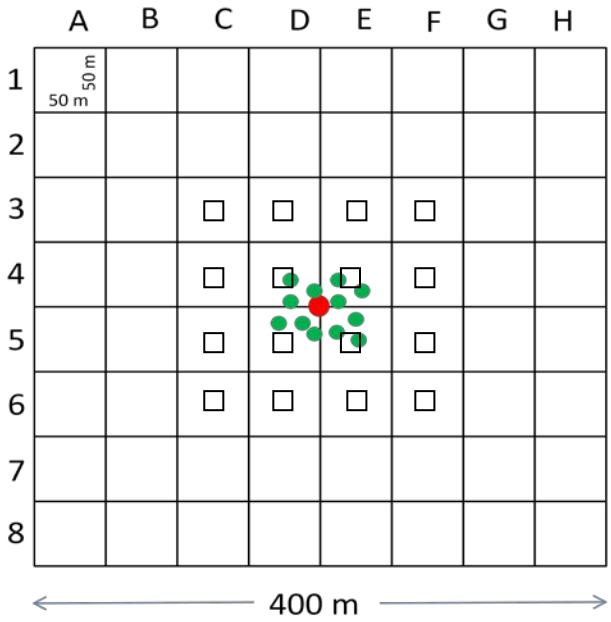
Location of release trees

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

Figure 1



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 (Appendix E) 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.

DATA ON RELEASE SITE CHARACTERISTICS

The information provided by cooperators on the Release Site Characterization Form (Appendix F) will provide data for researchers to assess site characteristics that may facilitate parasitoid establishment. This information includes: site location, some physical attributes, basal area of ash and other tree species, and DBH of ash trees in the plot. Mark off a 10 m X 10 m square in the center of each of sixteen 50 m × 50 m grid cells (Fig. 1, small, open square in each grid cell). For every tree > 4 cm DBH within each 10 m square, record the tree species, DBH, and crown position (open-grown, dominant, co-dominant, intermediate, suppressed). If the tree is an ash tree, also record the crown class (Appendix C) and whether or not there are symptoms of EAB (i.e., exit holes, epicormic shoots, woodpecker damage, bark splits) (Appendix A). These data will only be collected once for each site.

PARASITOID RELEASE

TIMING OF RELEASE

Spring Tetrastichus and Spathius releases: In more northerly 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 adults of 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 shortly after EAB adults begin to emerge. 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:

http://www.ipm.msu.edu/landreport/2004/EAB_tracking.pdf

http://www.emeraldashborer.info/files/handoutforpdf.pdf.

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.

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. Each release will consist of a specified number of female parasitoids previously exposed to male parasitoids for mating and an unspecified number of males.

Spring Tetrastichus and Spathius release: You will be shipped at least 200 *Tetrastichus* and 200 *Spathius* females every other week for 5 weeks beginning when 300 GDD have accumulated, for a total of at least 600 females released for each species.

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

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

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 adults in 16-oz plastic cups with mosquito screening on the lid or as developing pupae inside ash bolts. Ash bolts will contain a hanging device for easy mounting on the release tree. 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 filter paper.

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 already fitted with an eyelet screw in one end of each log. You will need some large nails with wide heads and a hammer to hang the logs. Place the nail through they eyelet screw and then pound it into the release trees to suspend the log. Another nail below the log to support it from the bottom may also be used. 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 ever harvested.

Complete the EAB Parasitoid Release Information Form (Appendix G) every time you release parasitoids. You may enter the information directly into a handheld GPS device or online at mapbiocontrol.org.

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. 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 consturct sentinel logs, yellow pan traps, or emergence tubes, please call one of the authors.

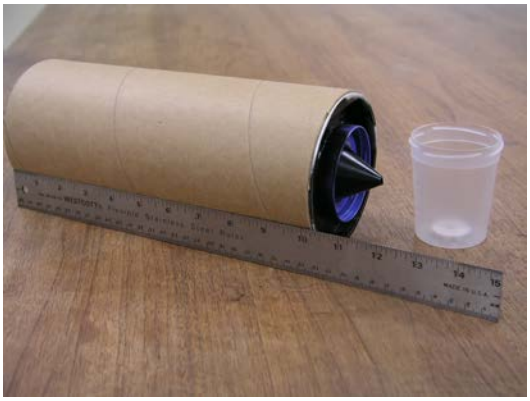
TREE FELLING AND DEBARKING OR EMERGING IN CARDBOARD TUBES

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, either search for parasitized EAB eggs on the bark (see Collection of EAB Eggs below) 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 (ca 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 (Appendix H).

***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 (Appendix H).

***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. Thus, rearing parasitoids from logs takes up considerable space and attention to detail, however, some may find it 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 (Appendix H).

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 not trap the EAB egg parasitoid *Oobius agrili.* 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 propylene glycol (non-toxic antifreeze) diluted with water
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 ~6-inch square piece of organdy (or similar thin fabric with mesh size <0.5 mm) for each YPT
2. one 50-ml plastic centrifuge tubes for each YPT
3. small kitchen strainer
4. pencils and paper
5. 70% ethanol

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.

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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. 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.

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. The traps should be placed on the central release trees and on nearby trees.

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

When should the YPTs be deployed? Deploy YPTs at EAB biocontrol release sites the year following the last larval parasitoid releases. The adult parasitoids fly throughout the spring, summer, and early fall. However, because their populations are highest in late summer and early fall; it is most efficacious to sample for parasitoids using the YPTs during the first two weeks of August and first two weeks of September.

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.

How is the insect sample collected from the YPT? After locating the YPT in the field, drape the piece of organdy over the kitchen strainer, and strain the contents of the trapping-bowl by pouring the liquid through the strainer, letting it drain onto the ground. Then, gently wrap the specimens in the piece of fabric and place it into a 50-ml plastic centrifuge tube with an ID label. Use pencil on paper to write the label (include state, YPT-ID number, and date). Fill each tube with 70-95% ethanol (EtOH) and store samples in the refrigerator.

What do I do with these samples? EtOH is flammable and only very small quantities can be shipped without requiring special handling. Pour off as much of the EtOH as possible from each tube without touching the sample, and seal the cap tightly. The residual ethanol remaining in each sample will be sufficient to preserve the insects during shipping. Send the samples by overnight shipping to Rearing Facility Manager. Enter recovery data (Appendix H) 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 3rd- or 4th-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. Retreive 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.

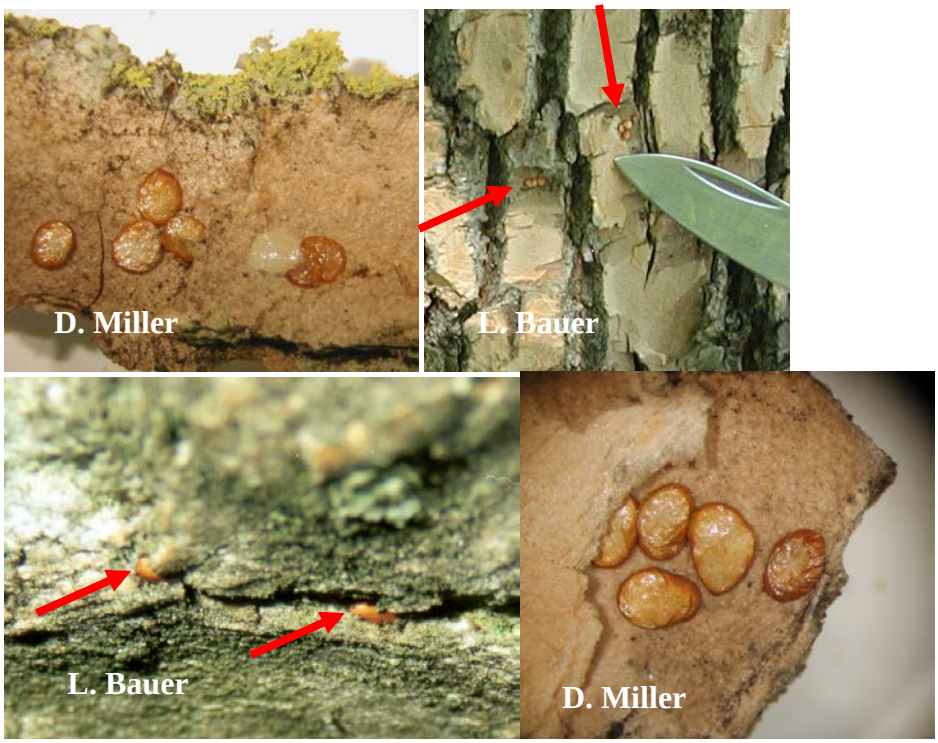
COLLECTION OF EAB EGGS

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,

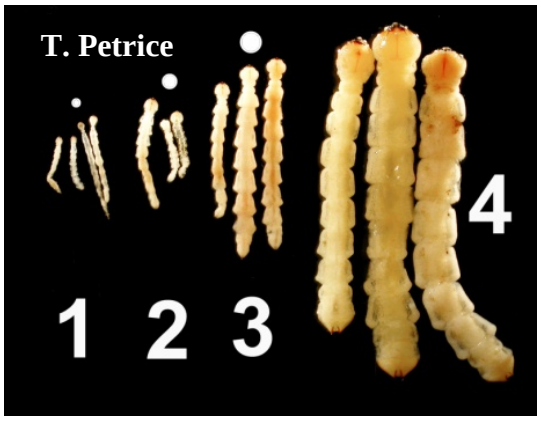
Oobius populations tend to be highest during the fall (through November). EAB eggs are laid in bark crevices or under bark flaps, so use a utility or pocket knife to gently lift off layers of the bark to find eggs. A headband magnifier, such as an Optivisor®, or magnifying reading glasses can help the 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.

EAB Life-Stages

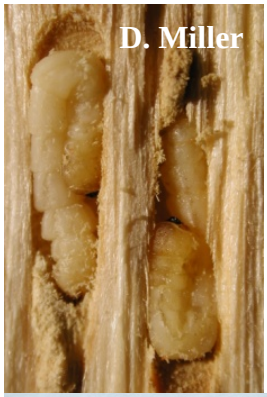
EAB eggs (newly laid egg is white, clutches of eggs that were under bark flakes, single eggs in bark crevices)

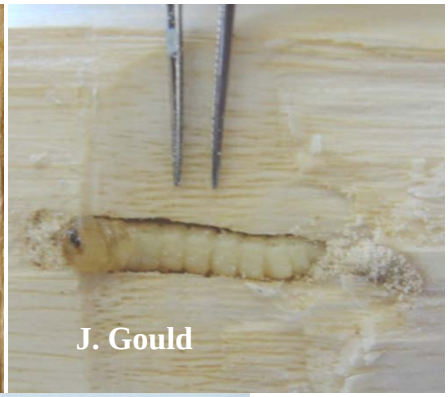


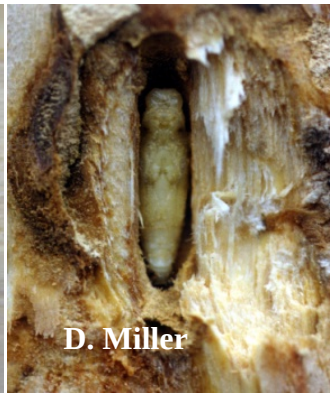
EAB larvae (1-4 indicates the four instars)



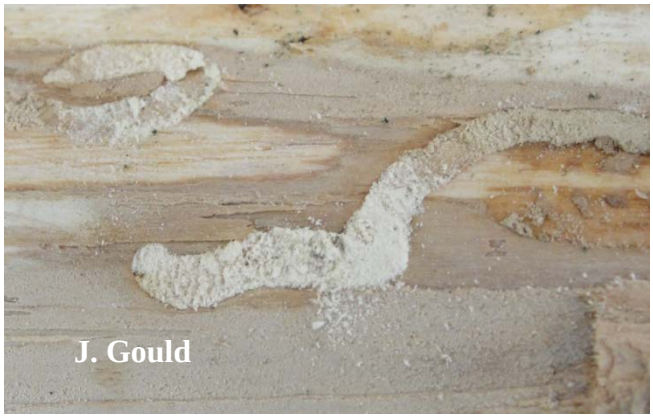
EAB J-shaped larvae, pre-pupa, pupae



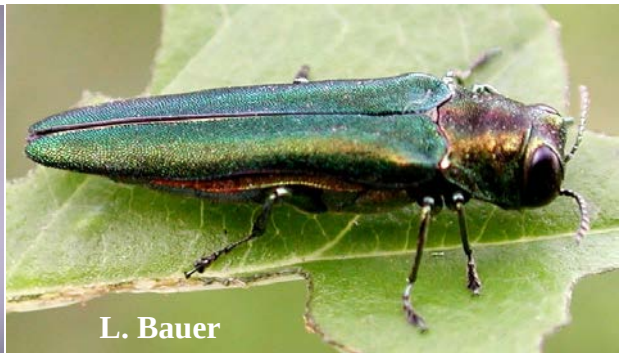




External signs of EAB overwintering chamber under the bark. The photo on the left shows the EAB gallery filled with light colored frass and the photo on the right shows the exits to three overwintering chambers, each with 2 holes filled with frass.



EAB Adult



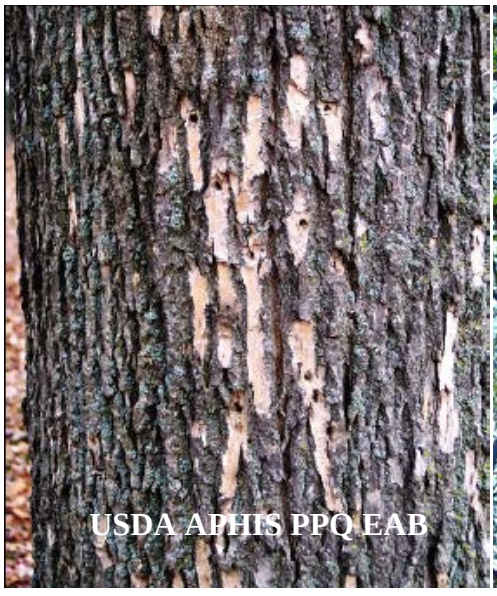
Signs of EAB infestation

Thinning Ash Crowns

Epicormic Shoots in Winter and Summer

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

Damage from Woodpecker Feeding



D-shaped exit holes

Larval Galleries

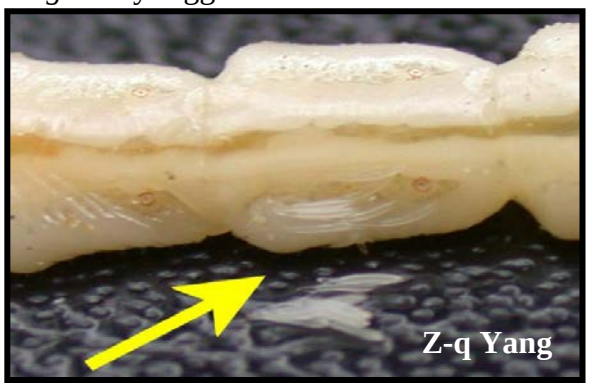
Additional photos and specific morphological and physiological information can be found in the EAB Program Manual at:

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

Life stages of EAB Parasitoids

Spathius agrili

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



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

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

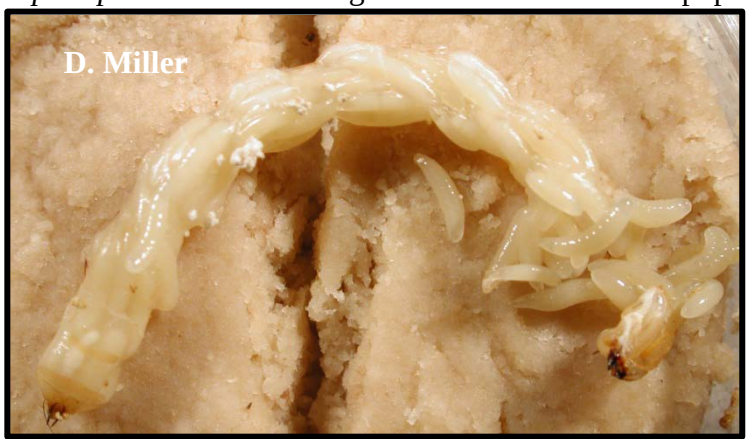
Female *S. agrili* lay eggs through ash bark onto an EAB larva.

Tetrastichus planipennisi

Immature T. planipennisi larvae inside EAB

Mature *T. planipennisi* larvae inside an EAB larva

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.

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.

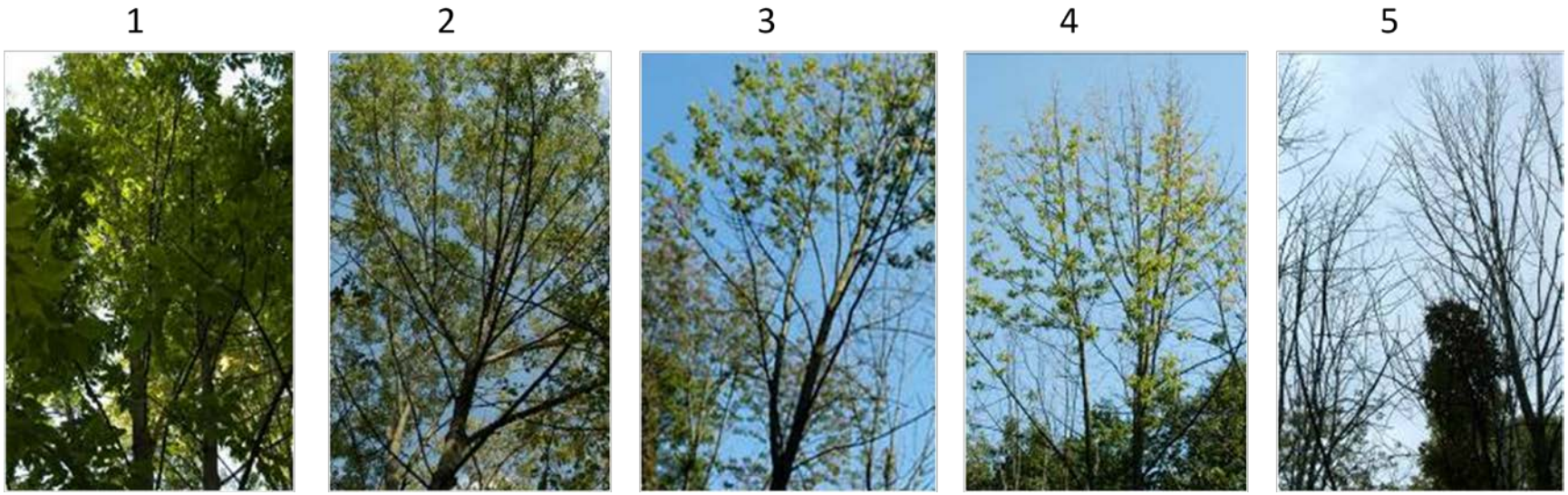
O. agrili female parasitize EAB eggs laid on ash bark.


D. Miller

Appendix C – Crown Condition of Ash Trees

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

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



Biological Control Pre-release Site Assessment

Collection of these data are required to determine the suitability of a site for release of biological control organisms to aid in the control of populations of emerald ash borer. Use the GPS unit to collect the data or enter the data online (www.mapbiocontrol.org). If the GPS is used it must be synchronized after data collection. It is not necessary to send a paper copy to the Rearing Facility Manager.

Date:_____
State: _____
Site Name: _____
Site Location: _____
Latitude: _____
Longitude: _____
Size of Wooded Area (in acres): _____
Percentage Ash (estimated) (circle one): <25% 25% 50% 75% 100%
Overstory composition:
Dominant species: _____
2nd Dominant species:_____
3rd Dominant species:_____
EAB Density (circle one): Low Medium High

Notes:
_____
_____
_____
_____
_____
_____

Program Contact Information:

Name: _____
Position: _____
Affiliation: _____
Address:_____
_____
_____
Office phone: _____
E-mail: _____

Release Site Contact Information

Site Ownership: _____

Site Contact: _____

Site Contact Phone No.: _____

Instructions for completing the Pre-Release Site Assessment

Date: Date on which assessment was conducted.

State: Enter the state in which the site is located.

Site Name: Each site must have a unique name. The spelling of the site should always be the same.

Site Location: Please include political entity location identity where parasitoids will be released (state, county, municipality, and name of park or road if applicable). An example might be "Archbald, OH, Woodland Park, off Rte. 66 south of town".

Latitude and Longitude: Include GPS coordinates in decimal/degree format.

Specific site information

Size of Wooded Area: Record the number of acres of the main release area. Do not include acreage of areas without ash or connecting corridors. You can estimate the size of the wooded area using the polygon tools on Google Earth or ArcGIS Explorer. You can enter these data at a later date by editing the data point on the GPS unit.

Percentage Ash: Estimate whether the percentage of ash in the stand falls into one of the following categories: 0-25%, 26-50%, 51-75%, 76-100%.

Overstory composition

Dominant species: Record the dominant species in the upper canopy.
2nd dominant species: Record the second most common overstory tree species.
3rd dominant species: Record the third most common overstory tree species.

EAB Density: Assess the density of the EAB infestation using Low, Medium, or High as a descriptor.

Low Density: EAB present but very difficult to find. Nearly 100% of ash trees are healthy.

Medium Density: Trees are beginning to show signs of EAB infestation (epicormic shoots, woodpecks, bark splits, emergence holes) but >75% of the trees are healthy and show no signs of EAB.

High Density: >25% of the trees show signs of EAB infestation.

Notes: Please include additional relevant information about this release site.

Program Contact Info:	Please print name, affiliation, and contact information for the person who is overseeing parasitoid release and data collection.
Site Ownership:	Record the entity or individual who retains title to the release site real estate.
Site Contact:	Record the individual's name and title designated as the site contact.
Site Contact Phone No.:	Record the site contact's phone number. Please note, if known, whether it is a cell phone or office number.

Release Tree Characteristics

For each of the 12 release trees record the following data in the GPS unit or online (www.mapbiocontrol.org). It is not necessary to send a paper copy to the Rearing Facility Manager.

State:_____

Site:_____

Tree Number:_____

GPS Coordinates:_____

DBH:_____

Ash Species:_____

Crown Class (1-5):_____

Lower half of Tree

Number of epicormic shoots: 0 1-10 11-20 21+

EAB exit holes: Yes No

Bark splits: Yes No

Woodpecker feeding damage present: yes no

Upper half of Tree

Number of epicormic shoots: 0 1-10 11-20 21+

Woodpecker feeding damage present: yes no

RELEASE TREE CHARACTERISTICS

State: Enter the state in which the site is located.

Site Name: Each site must have a unique name. The spelling of the site should always be the same every time data are entered.

Tree Number: Record the number on the tree tag.

GPS coordinates: Record the latitude and longitude of each tree in decimal degrees.

DBH: Record the diameter of each of the 12 release trees at breast height (4.5 feet or 1.37 m above the forest floor).

Ash Species: Record which species of ash you are sampling.

Crown Class: Use the 5 point crown class rating (Appendix C) to rate each tree.

Epicormic shoots: Epicormic shoots are sprouts that emerge from dormant buds along the trunk or branch of a tree. 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 the number of epicormic shoots on the main trunk of each tree in the upper and lower half of the tree. If there are a lot of epicormic shoots you may need to estimate rather than count.

Number of EAB exit holes: Record whether or not EAB exit holes are present on the lower half of the tree.

Number of bark splits: Record whether or not bark splits are present on the lower half of the tree.

Woodpecker Feeding Damage: Woodpeckers feed on EAB in or under the bark and leave a characteristic hole in the bark (Fig 4). They also create "scaling" (Fig 5), which are lighter patches where the bark has been removed by foraging woodpeckers. Record whether woodpecker feeding is observed on the upper or lower half of the trunk

Release Site Characterization

GENERAL SITE INFORMATION Use the GPS unit to collect data or enter the following data online (www.mapbiocontrol.org). It is not necessary to send a paper copy to the Rearing Facility Manager.

Date:_____

State: _____

Site Name: _____

Latitude and Longitude: _____

PHYSICAL SITE CHARACTERISTICS

Elevation: _____**Aspect:** _____

Topographic position:
Upper slope Mid slope Lower slope Level

Flooding: Dry all Year Seasonally Wet Wet all Year

Degree of Isolation: Isolated Connected to other Woodlots

Comments:

FOREST TYPE AND PERCENTAGE ASH

Fill out the following for all the trees > 4 cm DBH in each of the sixteen 10 m sampling circle.

State:______________________________ Site Name:____________________________

Grid Cell Column	Grid Cell Row	Tree Number	Species	DBH	Crown Position	Crown Class if ash	EAB signs if Ash

Instructions for completing the Release Site Characterization

GENERAL SITE INFORMATION

Date: Date on which characterization is accomplished.

State: Enter the state in which the site is located.

Site Name: Each site must have a unique name. The spelling of the site should always be the same every time data are entered.

Latitude and Longitude: Include GPS coordinates in decimal/degree format.

PHYSICAL SITE CHARACTERISTICS

Elevation: Actual elevation above mean sea level should be recorded from either GPS unit or from USGS Quadrangle map of the area.

Aspect: Record the azimuth of the direction of slope. The azimuth is the horizontal angle measured clockwise from true north. For example, moving clockwise on a 360° degree circle, a point due east would have an azimuth of 90°, south 180°, and west 270°.

Topographic position: Circle the description of the topography at the release epicenter.

Flooding: Record whether the soil at the site is covered by water all year, part of the year (seasonal flooding), or never.

Degree of Isolation: Circle one. Is your site isolated from other wooded areas by non-wooded land or is it connected to other woodlots by corridors containing ash trees?

FOREST TYPE CHARACTERISTICS.

Record the following for every tree > 4 cm DBH in the sixteen 10 x 10 m square plots.

Grid Cell Column: Record which column of grid cells you are sampling (A-H).

Grid Cell Row: Record which row of grid cells you are sampling (1-8).

Tree number: Number the trees within a cell sequentially. Order does not matter.

Species:	Record the species of the tree (See Appendix I for a list of species).
DBH:	Record the diameter of the tree at breast height (4.5 feet or 1.37m above the ground)
Crown Position:	**Open grown:** A tree growing in an open area; receives light from above and all sides **Dominant:** Crown occurs above all other crowns; receives light from above and upper sides **Co-dominant:** Crown occurs with other crowns forming the general level of the crown canopy; receives light from above but little from the sides **Intermediate:** Crown is below dominant and co-dominant level but extends up into the lower canopy; receives light from above but none from the sides **Suppressed:** Crown is overtopped by all other trees and receives no direct light from above or from the sides
Crown Class:	Use the 5 point crown class rating system (Appendix C) if the tree is an ash tree.
Signs of EAB:	Record if the ash tree shows signs of EAB infestation including exit holes, epicormic shoots, woodpecker feeding damage, or bark splits.

EAB Parasitoid Release Information

Enter these data in a GPS unit or online (www.mapbiocontrol.org) every time parasitoids are released. It is not necessary to send a paper copy to the Rearing Facility Manager.

State: _____
Site Name: _____
Release Date: _____
Time of Day: _____
Weather conditions: Sunny Partly Cloudy Cloudy fog light rain mod rain heavy rain thunderstorms
Approximate wind speed: Light Moderate Strong
Ambient Temperature (ºF): _____
Number Females Released:
Species: *Oobius*_____ *Spathius*_____ *Tetrastichus*_____
Other _____

Release done by:
Name: _____
Phone: _____

Instructions for completing the EAB Parasitoid Release Information Form

State: Enter the state in which the site is located.

Site Name: Each site must have a unique name. The spelling of the site should always be the same every time data are entered.

Release Date: The date that the parasitoids were released in the field.

Time of Day: Time when parasitoids are released onto the trees.

Weather conditions: Circle the approximate weather conditions at the time of release.

Approximate wind speed: Circle the approximate wind conditions at the time of release.

Ambient Temperature (^{0}F): Record the actual air temperature at the time of release in degrees Fahrenheit.

Number Released: Record the number of female parasitoids released. Record numbers by species.

Site data collection completed by: Please print name and phone number.

Parasitoid Establishment Sampling Data

Data on the locations where the samples are collected can be entered in the field using a handheld GPS unit. Data on recovery of parasitoids will need to be entered later after parasitoids have been recovered.

State: Enter the State where recovery sampling took place.

Site Name: Each site must have a unique name. The spelling of the site should always be the same every time data are entered.

Collector's Name: Enter the name of the person responsible for collecting and processing the samples.

Collector's Phone: Phone number for contacting the collector.

Recovery Method: Yellow Pan Trap Tree Debarking Logs/Bark in Tubes
Egg Sentinel Logs Larval Sentinel Logs Egg Collection

Different data need to be entered for each recovery method. When you click on one of the recovery methods on the drop down menu on mapbiocontrol, you will be taken to a page specifically designed to record data for each of the recovery methods.

Yellow Pan Traps

Trap Number: Create an alphanumeric numbering system for your traps.

Trap Location: GPS coordinate and/or grid cell number where the trap was deployed.

Date Trap Deployed: The date when the yellow pan traps were placed in the field.

Date Trap Sampled: The date when the insects are removed from the pan trap.

Tree DBH: Record the diameter of the tree at breast height (4.5 feet or 1.37m above the ground). Specify whether the DBH was recorded in inches or cm.

Tree Crown Class: Use the 5 point crown class rating system (Appendix C).

Were Samples Shipped: Enter yes or no as to whether there were any insects caught and shipped for identification.

The following information will be entered by personnel who are qualified to positively identify the exotic parasitoids.

Number *S. agrili*: The number of positively identified *S. agrili* present in the sample.

Name of *S. agrili* identifier: The name of the person who positively identified the parasitoid as *S. agrili*.

Number *T. planipennisi*: The number of positively identified *T. planipennisi* present in the sample.

Name of *T. plan.* identifier: The name of the person who positively identified the parasitoid as *T. planipennisi*.

Number *O. agrili*: The number of positively identified *O. agrili* present in the sample.

Name of *O. agrili* identifier: The name of the person who positively identified the parasitoid as *O. agrili*.

Tree Debarking

Tree Number: Create an alphanumeric numbering system for your trees.

Tree Location: GPS coordinate and/or grid cell number where the tree was felled.

Date Tree Cut: The date when the tree was cut in the field.

Date Tree Debarked: The date when the tree was debarked and sampled.

Tree DBH: Record the diameter of the tree at breast height (4.5 feet or 1.37m above the ground). Specify whether the DBH was recorded in inches or cm.

Tree Crown Class: Use the 5 point crown class rating system (Appendix C). These data will have to be taken in the summer before tree cutting (if possible) because trees will not have leaves in winter.

 # Clutches Gregarious: Parasitoids Enter the number of clutches of gregarious cocoons or larvae that are collected and shipped for rearing and identification. It is important to enter zeros.

 # Solitary Parasitoids: Enter the number of solitary cocoons or larvae that are found. These should be counted but not sent for identification.

 # Live EAB: Enter the number of live EAB larvae, pre-pupae, or pupae that are found. These should be counted but not sent for identification. It is important to enter zeros.

The following information will be entered by personnel who are qualified to positively identify the exotic parasitoids.

Number *S. agrili*: The number of positively identified *S. agrili* present in the sample.

Name of *S. agrili* identifier: The name of the person who positively identified the parasitoid as *S. agrili*.

Number *T. planipennisi*: The number of positively identified *T. planipennisi* present in the sample.

Name of *T. plan.* identifier: The name of the person who positively identified the parasitoid as *T. planipennisi*.

Number *O. agrili*: The number of positively identified *O. agrili* present in the sample.

Name of *O. agrili* identifier: The name of the person who positively identified the parasitoid as *O. agrili*.

Logs or Bark in Tubes

Tube Contents: Log or Bark. Enter whether you have put bark samples or entire logs in the rearing tubes.

Tree Number: Create an alphanumeric numbering system for your trees.

Tree Location: GPS coordinate and/or grid cell number where the tree was felled.

Tree DBH: Record the diameter of the tree at breast height (4.5 feet or 1.37m above the ground). Specify whether the DBH was recorded in inches or cm.

Number Parasitoids: Shipped Enter the number of parasitoids that were collected and shipped for identification.

The following information will be entered by personnel who are qualified to positively identify the exotic parasitoids.

Number *S. agrili*: The number of positively identified *S. agrili* present in the sample.

Name of *S. agrili* identifier: The name of the person who positively identified the parasitoid as *S. agrili*.

Number *T. planipennisi*: The number of positively identified *T. planipennisi* present in the sample.

Name of *T. plan.* identifier: The name of the person who positively identified the parasitoid as *T. planipennisi*.

Number *O. agrili*: The number of positively identified *O. agrili* present in the sample.

Name of *O. agrili* identifier: The name of the person who positively identified the parasitoid as *O. agrili*.

Egg Sentinel Logs

Log Number: Create an alphanumeric numbering system for your sentinel logs.

Deployment Location: Record the GPS coordinate and/or grid cell number where the ESL was deployed.

Tree DBH: The DBH (specify cm or inches) of the tree on which the sentinel log was placed.

Tree Crown Class: Use the 5 point crown class rating system (Appendix C).

Date Log Deployed: Enter the date when the sentinel log was placed in the field.

Date Log Recovered: Enter the date when the sentinel log was removed from the field.

Number of Eggs/Log: Record the number of EAB eggs on the log before deployment.

Number Eggs Shipped: Record the number of EAB eggs shipped for rearing and identification.

The following information will be entered by personnel who are qualified to positively identify the exotic parasitoids.

Number *O. agrili*: The number of positively identified *O. agrili* present in the sample.

Name of *O. agrili* identifier: The name of the person who positively identified the parasitoid as *O. agrili*.

Larval Sentinel Logs

Log Number: Create an alphanumeric numbering system for your sentinel logs.

Deployment Location: Record the GPS coordinate and/or grid cell number where the LSL was deployed.

Tree DBH: The DBH (specify cm or inches) of the tree on which the sentinel log was placed.

Tree Crown Class: Use the 5 point crown class rating system (Appendix C).

Date Log Deployed: Enter the date when the sentinel log was placed in the field.

Date Log Recovered: Enter the date when the sentinel log was removed from the field.

Number of Larvae/Log: Record the number of EAB larvae inserted into the log.

 # Clutches Gregarious: Parasitoids Enter the number of clutches of gregarious cocoons or larvae that are collected and shipped for rearing and identification. It is important to enter zeros.

 # Solitary Parasitoids: Enter the number of solitary cocoons or larvae that are found. These should be counted but not sent for identification.

 # Live EAB: Enter the number of live EAB larvae, pre-pupae, or pupae that are found. These should be counted but not sent for identification. It is important to enter zeros.

The following information will be entered by personnel who are qualified to positively identify the exotic parasitoids.

Number *S. agrili*: The number of positively identified *S. agrili* present in the sample.

Name of *S. agrili* identifier: The name of the person who positively identified the parasitoid as *S. agrili*.

Number *T. planipennisi*: The number of positively identified *T. planipennisi* present in the sample.

Name of *T. plan.* identifier: The name of the person who positively identified the parasitoid as *T. planipennisi*.

Collection of Naturally Occurring Eggs

Tree Number: Create an alphanumeric numbering system for your trees.

Tree Location: GPS coordinate and/or grid cell number where the tree was felled.

Tree DBH: Record the diameter of the tree at breast height (4.5 feet or 1.37m above the ground). Specify whether the DBH was recorded in inches or cm.

Tree Crown Class: Use the 5 point crown class rating system (Appendix C).

Date: The date when the eggs were sampled.

Number Eggs Shipped: Enter the number of EAB eggs that were found and shipped for rearing and identification.

The following information will be entered by personnel who are qualified to positively identify the exotic parasitoids.

Number *O. agrili*: The number of positively identified *O. agrili* present in the sample.

Name of *O. agrili* identifier: The name of the person who positively identified the parasitoid as *O. agrili*.

LIST OF TREE SPECIES

alder
ash, black
ash, green
ahs, white
ash, other,
aspen/poplar
bassword
beech
birch, yellow
birch, other
black locust
boxelder
cedar
cherry
cottonwood
elm
fir
hemlock
hickory
maple, red
maple, sugar
maple, other
mountain ash
oak, black/red
oak, white
oak, other
pine, jack
pine, red
pine, white
pine, other
spruce
sycamore
tamarack/eastern larch
walnut
willow
unknown broadleaf
unknown conifer

Appendix J – Helpful Links

mapBioControl (to request parasitoids and enter release data)

www.mapbiocontrol.org

e-authentication application

http://www.aphis.usda.gov/permits/eauth_epermits.shtml

e-permits

http://www.aphis.usda.gov/permits/learn_epermits.shtml

Growing Degree Days

http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/cdus/degree_days/grodgree.txt

http://uspest.org/US/

For MI: http://www.enviroweather.msu.edu/homeMap.php

Timing of EAB Emergence

http://www.ipm.msu.edu/landreport/2004/EAB_tracking.pdf

http://www.emeraldashborer.info/files/handoutforpdf.pdf

General EAB Information

EAB Program Manual

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

APHIS Emerald Ash Borer Home Page

http://www.aphis.usda.gov/plant_health/plant_pest_info/emerald_ash_b/index.shtml

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/