United States Department of Agriculture

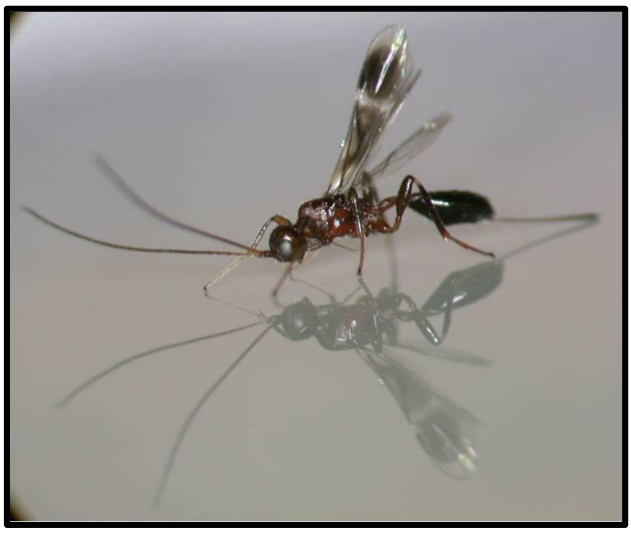
Animal and Plant Health Inspection Service

Agricultural Research Service

US Forest Service

Cooperating State Departments of Agriculture

Emerald Ash Borer Biological Control Release and Recovery Guidelines



Emerald Ash Borer, *Agrilus planipennis* (Fairmaire), Biological Control Release and Recovery Guidelines 2016

Prepared by:

Juli S. Gould, Entomologist
USDA APHIS PPQ CPHST Laboratory
Juli.R.Gould@aphis.usda.gov
508-563-0923

Leah S. Bauer, Research Entomologist
USDA Forest Service, Northern Research Station
lbauer@fs.fed.us
517-884-8059

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

ACKNOWLEDGEMENTS

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

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

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

Cite this report as follows: USDA–APHIS/ARS/FS. 2016. Emerald Ash Borer Biological Control Release and Recovery Guidelines. USDA–APHIS–ARS-FS, Riverdale, Maryland.

CONTENTS

SECTION	PAGE
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
INTRODUCTION	1
Brief History of EAB in North America	1
Life Cycle of EAB	2
Damage and Signs of Infestation	3
Economic Consequences of EAB Infestations	4
Host Range of EAB	5
BIOLOGICAL CONTROL OF EMERALD ASH BORER	6
Biology of EAB Biocontrol Agents	6
Oobius agrili	6
Spathius agrili	7
Tetrastichus planipennisi	7
Spathius galinae	7
Rearing EAB Parasitoids	8
Project Status	9
Preparation for Parasitoid Release	10
FIELD RELEASE	10
Outline of Procedures for EAB Biocontrol Releases	10
Release Site Selection	12
Pre-Release Site Assessment	15
Pre-Release Site Preparation	17
Collection of Data from Release Trees	18
RELEASE OF PARASITIOIDS	19
Which Species to Release	19
Timing of Release	20
Number and Frequency of Releases	22
Requesting Parasitoids	23
Receipt of Parasitoids	24
Care of Parasitoids if Release is Delayed	25
Transporting Parasitoids to Field Sites	26
Release of Parasitoids	26
Enter Release Data	27
EVALUATING PARASITOID ESTABLISHMENT	28
Tree Felling and Debarking	30
Debarking	31
Yellow Pan Traps	33
ENTER RECOVERY DATA	38

APPENDICES

Appendix A – EAB Life Stages and Damage A-1

Appendix B - Parasitoid Life Stages B-1

Appendix C – Crown Condition of Ash Trees C-1

Appendix D – Helpful Links D-1

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 March 2016, EAB infestations were known in twenty-five states (Arkansas, Colorado, Connecticut, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, and Wisconsin) and two Canadian provinces (Ontario and Québec). At present, the most sustainable and long-term approach to reducing EAB populations and conserving ash in forested areas of North America is biological control.

LIFE-CYCLE OF EAB

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

Adults

EAB adults begin to emerge from ash trees after the accumulation of 400-500 growing degree days (GDD) base 50°F. Peak adult activity occurs at ~1,000 GDD. After emergence, adults fly into the ash canopy where they feed on leaves throughout their lives. EAB adults start mating one week after emergence, and females begin laying eggs 2-3 weeks later. In the field, EAB adults are readily observed mating and egg-laying on ash trees on warm, sunny afternoons. The adults of both sexes are strong fliers.

Eggs

A female EAB may lay >200 eggs in her lifetime, depositing them individually or in groups on the bark along the trunk and portions of 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 two to three weeks.

Larvae

Newly hatched larvae bore through the bark to the phloem and outer layer of new sapwood where they feed until the weather gets too cold in the fall. There are four stages of larval development (instars) (Appendix A). As they feed, the larvae create serpentine galleries

filled with frass (excrement), which enlarge in width as the larvae grow (Appendix A). Larvae are creamy white, and dorso-ventrally flattened (Appendix A). When fully mature, 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 posterior pair of small brown structures called urogomphi (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 darkens as they develop to the adult stage (Appendix A). To emerge from ash trees, adults chew D-shaped exit holes (Appendix A) through the bark and are 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 population density increases, the movement of nutrients through the phloem is disrupted and evidence of tree stress increases such as yellow foliage on dying branches, dead branches, small leaves, thinning crowns, and epicormic shoots (Appendix A). Woodpeckers feed on EAB larvae living under the bark of trees. Field observations suggest woodpecker feeding is one of the best indicators of early EAB infestation with the most obvious symptoms including bark scaling (removal of bark flakes) 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 forest ecosystems in North America. The 16 native species of ash, some with limited distributions in North America, are now threatened by EAB.

In addition to its value to the timber industry and the forest ecosystem, ash was one of the most popular landscape trees because of its tolerance of a range of environmental conditions and resistance to 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 North America, EAB attacks ash species in the genus *Fraxinus*, including but not limited to green ash (*F. pennsylvanica*), white ash (*F. americana*), black ash (*F. nigra*), pumpkin ash, (*F. profunda*), and blue ash (*F. quadrangulata*). In China, native ash species, including Chinese ash (*F. chinensis*) and Manchurian ash (*F. mandshurica*), are less susceptible to EAB than North American species commonly planted in China such as velvet ash (*F. velutina*) and green ash (*F. pennsylvanica*). In 2014, EAB was observed attacking North American white fringetree, *Chionanthus virginicus* L. in Dayton, Ohio. However, the impact of EAB on white fringetree is not yet well understood.

BIOLOGICAL CONTROL OF EMERALD ASH BORER

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

BIOLOGY OF EAB BIOCONTROL AGENTS

Oobius agrili parasitizes up to 60% of EAB eggs laid during the summer in some areas of China. Tiny female *Oobius* accomplish this by searching the bark of ash trees for EAB eggs, 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 up to ~80 EAB eggs during its lifetime. *Oobius* spend the winter as larvae inside EAB eggs and emerge as adults the following spring.

Spathius agrili parasitizes up to 90% of EAB larvae in ash trees east of Beijing in Tianjin, China, where the climate is relatively mild. 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 following summer.

Tetrastichus planipennisi is another larval parasitoid of EAB collected 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 up to130 *Tetrastichus* adults. They survive the winter as larvae inside their host or host gallery under the bark of ash trees (Appendix B).

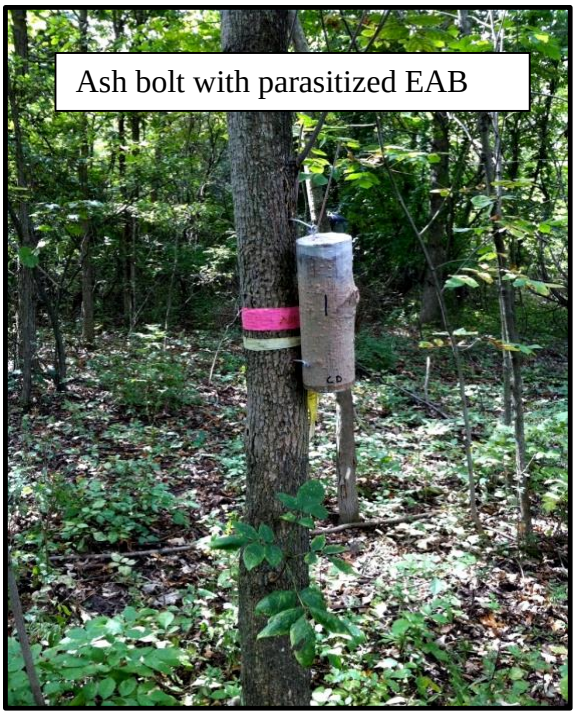
Spathius galinae has a life-history that is similar to *S. agrili*, but it was collected from more northerly locations in Russia and South Korea. In Russia, parasitism of EAB larvae is as high as 63% in green ash trees, which were imported to Russia from North America. Russia is more climatically similar to the northern U.S. than Tianjin, China, where *S. agrili* was collected, and it is hypothesized that *S. galinae* will have a better chance of establishing in more northern states. This species of *Spathius* will fill an important niche in northern states where *S. agrili* is not establishing and where *T. planipennisi* is unable to attack EAB in large trees with thick bark. Females can produce up to 94 progeny over their lifetime (average of 47), and the clutch size averages 8 parasitoids per host.

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 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 allowed to emerge 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 (coffee filters). EAB eggs attached to coffee filters are then exposed to *Oobius* females, which parasitize the eggs. *Oobius* will be shipped as adults in plastic cups with solid caps or as mature pupae inside EAB eggs on paper held inside pill vials with screening (Oobinators). *Oobius* adults are released from the plastic cups by opening the lids, inverting the cup, and tapping it gently against the trunks of EAB-infested ash trees at release sites. *Oobius*

pupae are released by attaching the Oobinators to ash trees, with the screen-side down. The *Oobius* adults will emerge and disperse naturally.



The three species of EAB larval parasitoid, *S. agrili*, *S. galinae*, and *Tetrastichus*, are reared in small-diameter ash bolts in which EAB larvae are grown from eggs applied to the bark. When the EAB larvae are mature inside the ash bolts, they are exposed to either *Spathius* or *Tetrastichus* adults, which detect and parasitize EAB larvae feeding under the bark. Although some *Spathius* and *Tetrastichus* adults may be shipped in plastic cups, most of the larval parasitoids are shipped as mature pupae in small ash bolts. These bolts will be shipped with a small hole drilled through the top to provide a point of attachment to a release tree. Twine or zip ties are common materials used to attach release bolts.

PROJECT STATUS

In January 2009, a Biological Control Production Facility became operational in Brighton, MI. As of February 2016, this facility has reared and released over three million EAB parasitoids in twenty-two states and two Canadian Providences. Scientists have found that *Tetrastichus planipennisi* and *Oobius agrili* are establishing in multiple states, they are dispersing from the release sites, and percentage parasitism continues to increase. *Spathius*

agrili has not established in the northern states. *Spathius galinae* was discovered attacking EAB larvae in Korea and Russia, where the climate is colder than sites in China where *S. agrili* originated. Testing confirmed that *Spathius galinae* is quite host specific, and it was permitted for field release in 2015. A small number of releases have occurred in a few states, colonies were transferred to the rearing facility in fall 2015, and release of higher numbers is anticipated in 2016. Another species of *Oobius*, reared from EAB eggs collected in Russia, is being evaluated as an EAB biocontrol agent for some regions of the U.S.

PREPARATION FOR PARASITOID RELEASE

This section provides guidance for selecting parasitoid-release sites, collecting data on site characteristics, and releasing the parasitoids. For the EAB Biocontrol Program to monitor and evaluate the establishment of EAB parasitoids and the impact of EAB biocontrol, researchers and cooperators receiving parasitoids from USDA APHIS Biological Control Production Facility must agree to submit their release and recovery 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 recovered. Personnel can use a hand-held GPS device or computer (such as an iPad or iPhone) to collect data in the field, or data can be entered online. The data in the GPS or iPhone should be synchronized (uploaded to mapbiocontrol.org) with a central database for storage and analysis after every data collection occurrence.

FIELD RELEASE

OUTLINE OF PROCEDURES FOR EAB BIOCONTROL RELEASES

- **It is no longer necessary** to obtain release permits. The EAB Biocontrol Rearing Facility has permits to release all four parasitoid species in all of the states currently infested with EAB.

- **Select a release site** in an area with good access, high density of ash trees of various sizes, and 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. You can either use a GPS unit or the new iPad/iPhone app and synchronize to upload your data, or you can enter the data directly on the web site. 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. Only one release site in a general area needs to be sampled for parasitoid recovery.

- **Collect General Site Details and Physical Characteristics** using a GPS unit, iPad/iPhone or manually enter the information into the MapBioControl web site (www.mapbiocontrol.org).

- **Synchronize your GPS unit or iPhone.** If you are using a GPS unit or iPhone, the unit should be synchronized or uploaded to MapBioControl.org every time data are collected to prevent the loss of data.

- **Request Parasitoids for Release** at EAB.Biocontrol.Program@aphis.usda.gov once your site has been approved.

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

- **Release Parasitoids:** Release at least the minimum recommended number of parasitoids in the spring, mid-summer, and late summer in Year 1 and Year 2 (See Parasitoid Release Section for details). Enter Release data using the handheld GPS device or tablet or online at mapbiocontrol.org.

- **Assess Parasitoid Establishment:** Determine if the parasitoids are established in each general release area (not necessarily at each site) **at least one year following the Year 2 release (**Year 3 or more). This should be done during the fall, winter, or early spring (for bark sampling or log debarking) or late spring, summer, and early fall (for yellow pan traps). Several methods are available for parasitoid recovery, with the choice of method depending on the specific circumstances of each release site.

RELEASE SITE SELECTION

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

General Site Characteristics

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

Minimum Acreage

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

Relative Density of Ash

If possible, at least 25% of the trees should be ash, but a higher percentage of ash would be even better. The percentage of ash can be estimated as <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. Unless there are many young trees in the vicinity, stands with dead and dying ash trees are not appropriate as release sites because ash and EAB may decline 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).

During the spring and summer when the trees have leaves, the condition of ash trees can be visually ranked according to the five crown-condition classes illustrating typical EAB-induced decline; crown condition 1 is a healthy canopy, 2, 3, and 4 show increasing decline, and 5 is dead (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 conduct recovery activities over a period of three to five years. Keep in mind that it may take months to obtain permission or land-use permits from land owners or park managers.

PRE-RELEASE SITE ASSESSMENT

Prior to requesting parasitoids for release, we recommend collecting some preliminary data on site characteristics that will help the Biocontrol Rearing Facility Staff assess whether your site is appropriate for parasitoid release. General Site Details and Physical Details data can be collected with programmed GPS units or iPhone and can also be entered online at www.mapbiocontrol.org. When collecting data using a GPS device or iPhone, be sure that you are at the location where parasitoids will be released. Do not collect GPS coordinates next to the road. Ideally the parasitoids should be released in the center of the forest, or at least 100 m from the road or other non-forested areas. Once the data are collected and the GPS unit or iPhone is synchronized, Rearing Facility personnel can review the site and determine if the site is appropriate for release. The information provided, including location, size (number of acres or hectares), percentage ash, and EAB density will assist Rearing Facility personnel and state cooperators prioritize and select the best site(s) for parasitoid release.

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

- **Status:** Select "Proposed" because the site has not yet been approved.
- **State**
- **Date**

- **Site Name**
- **Site Location** (Enter general information such as county, town, park name, address, major roads, etc.)
- **Latitude** (dd.dddddd)
- **Longitude** (dd.dddddd)
- **Plot** (whether it is a release or control plot)
- **Type** (program or research)

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

General Details

- **Size of wooded area in acres** (you can use the measurement tools with Google Earth or ArcGIS Explorer)
- **% ash** (estimate 25%, 50%, 75%, 100%)
- **Dominant Tree Species**
- **2nd most Dominant Tree Species** (if applicable)
- **3rd most Dominant Tree Species** (if applicable)
- **EAB Density** (Low, Medium, High)

Low: EAB present but difficult to find.

Med: Trees are beginning to show signs of EAB infestation.

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

Physical Details

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

PRE-RELEASE SITE PREPARATION

General Plot Design and tree selection

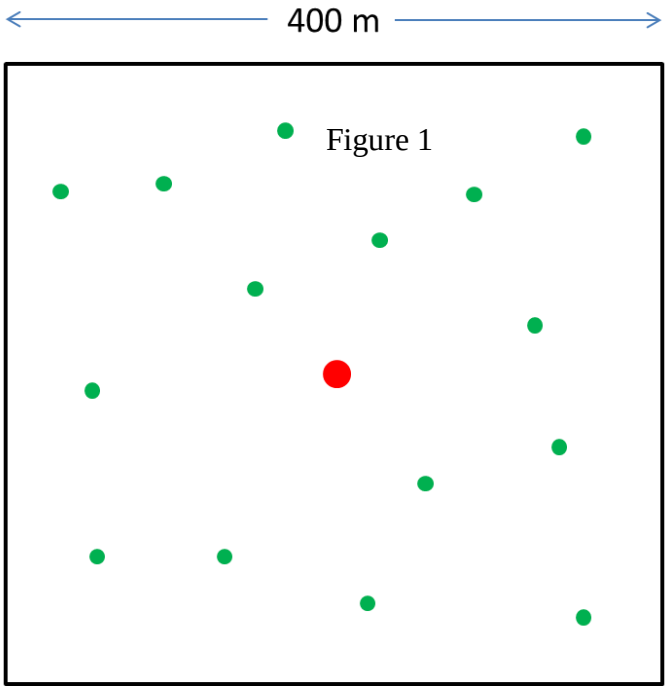


Figure 1

After a site is chosen for parasitoid release, locate the center of the release plot generally in the middle of the trees infested with EAB (Figure 1). Positive signs of EAB include woodpecker scaling and feeding, bark splits, epicormic shoots, poor crown condition, and/or EAB emergence holes. This will be the parasitoid-release epicenter. Generally, each plot will be 16 ha (400 m x 400m) surrounding the epicenter point, but it is not a problem if your site is long and narrow, for instance if it is along a stream. Figure 1 is

only one example of site shape. Select one tree 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.

Release trees should be large enough to support hanging logs containing parasitoids and should be spread throughout the plot (Figure 1, green dots). Flag all trees with brightly colored flagging so they are easier to find, and record the location of each tree using high resolution GPS. Flagging will not be provided by the Biocontrol Rearing Facility.

COLLECTION OF DATA FROM RELEASE TREES

Collect data for each of the release trees using a handheld GPS unit, tablet, 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.

RELEASE OF PARASITIODS

WHICH SPECIES TO RELEASE

Prior to 2012, the EAB Biocontrol Program provided *S. agrili, T. planipennisi, and O. agrili* to each state for release upon request at suitable sites. Releases of *Tetrastichus* and *Oobius* will continue in all states. *Spathius agrili*, however, is not surviving adequately in northern regions. Therefore, the EAB Biological Control Program will now only provide *S. agrili* for programmatic releases at sites 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. At this point in time, release and recovery efforts of *S. agrili* in more southerly states such as Kentucky, Missouri, Tennessee, Virginia, and West Virginia are too recent for establishment confirmation. 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.

Initial priorities for release of *S. galinae* will be at sites north of the 40th parallel. General locations where EAB parasitoids have already been released are still appropriate for releases of *S. galinae* because this parasitoid has a long ovipositor and can attack EAB in much larger trees than *T. planipennisi*, even if *T. planipennisi* has already established. Care must be taken, however, to select sites where there are still a substantial number of living ash trees so that the trees do not die before *S. galinae* can establish.

TIMING OF RELEASE

Larval parasitoids should be released when late instar EAB larvae (third and preferably fourth instar larvae) are present in the field. Making firm recommendations on release timing is difficult because EAB larval development is variable and depends on factors such as when the eggs were laid, temperature, and ash tree health. In addition, *Oobius* should be released when EAB eggs are present. However, EAB eggs are very small and nearly impossible to find in the field, and EAB larvae are under ash bark and not always accessible without felling trees. If you can scrape ash bark and determine EAB larval development to inform release timing; that is preferred. Otherwise, the guidelines below, coupled with the long lives of the parasitoids, the asynchrony of EAB development, and spreading releases out

over many weeks should help ensure that the proper stages of EAB eggs and larvae are present for parasitization.

Spring larval parasitoid 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 (not J-larvae in overwintering chambers) needed for parasitism by *S. agrili* and *T. planipennisi* are present during spring, summer, and fall. If you do not have the ability to confirm the presence of late instar EAB larvae, begin releasing the larval parasitoids when 300 growing degree days (GDD) base 50ºF have accumulated in your area. Growing degree day accumulations and forecasts can be found at http://uspest.org/US/. South of the 40th parallel, unless field sampling confirms the presence of late instar EAB larvae; do not conduct a spring release of larval parasitoids.

Summer Oobius releases: As soon as you see EAB adults in the field (on traps) you should initiate the process of obtaining and releasing *O. agrili*. If you are not trapping EAB, initiate releases when ~800 GDD have accumulated. In northern areas or areas with late emergence of EAB adults, *Oobius* releases may continue through August. A helpful handout on the timing of EAB emergence is online at:

http://extension.psu.edu/plants/green-industry/news/2012/resource-for-tracking-growing-degree-days-emerald-ash-borer

Late summer/Fall release of larval parasitoids: All three 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 most abundant in mid-summer (July to August) whereas in more northern states larvae may remain available for parasitization into the fall (September to October). Begin late summer/fall releases when sampling reveals the presence of third and/or fourth instar EAB larvae or 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

In theory, a higher number and frequency of parasitoids released increases the probability of establishing stable parasitoid populations. In reality, however, the number and frequency of parasitoid releases are often limited by the limited resources available for parasitoid production. 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 researchers have found that parasitoid establishment is more likely when more parasitoids are released in a given area. Each release will consist of a specified number of female parasitoids, although males are also included in shipments of adult parasitoids. 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 every other week for 5 weeks beginning when 300 GDD have accumulated, for a minimum of 600 females released for each species to be released sequentially three times. 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 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. For releases in southern states, if larval parasitoids were not released in the spring, release a minimum of 400 females per release occasion in the fall.

REQUESTING PARASITOIDS

Email all parasitoid requests to the EAB Biocontrol Program mailbox (EAB.Biocontrol.Program@aphis.usda.gov). If you have an emergency, please contact the Biological Control Release Coordinator - David Burt (810-844-2707) or the Facility Manager - Ben Slager (810-844-2704). You should enter data on the site characteristics and parasitoid releases into MapBioControl; however, the Request Tab for parasitoid requests is currently under development and should not be used until you are contacted by the Rearing Facility.

RECEIPT OF PARASITOIDS

Parasitoids are shipped by overnight delivery in a cardboard box, and should arrive by 10:30 AM at most locations. *Spathius* and *Tetrastichus* will be shipped either as developing pupae inside ash bolts or as adults in 16-oz plastic cups with screening on the lid. Ash bolts will have a hole drilled through the log as a point of attachment for mounting on release trees. Twine and zip ties are commonly used attachment materials, but are not included in parasitoid shipments. Honey will be smeared on the screening as a source of food for the adult parasitoids in cups. Adult *Oobius* will be shipped in plastic cups with honey streaked on the walls of the cup, and sealed shut with a snap-top plastic lid lined with tissue paper. *Oobius* may also be shipped as pupae inside EAB eggs on paper held in small plastic vials that can be hung on ash trees at release sites. Small twist ties can be fixed to these containers for hanging on small diameter branches, but this apparatus can be attached to larger diameter branches with the twine or zip ties.

The parasitoids should be released soon after receipt. All parasitoids should be released on the day they are received. If you are unable to release parasitoids as scheduled because of personnel shortages or adverse weather conditions, contact the Biological Control Release Coordinator to arrange for a different shipping date. After arrival, transport the parasitoids in the container to the release site. *Oobius agrili* do not fly as far as *Tetrastichus* and *Spathius*, so their release cups should be spread throughout the stand to enhance their establishment and dispersal.

CARE OF PARASITOIDS IF RELEASE IS DELAYED

The parasitoids should be released the day of arrival, however, if there is an unforeseen delay caused by late delivery or unexpected weather conditions, place the cardboard containers in an air-conditioned room, unseal and open each box and internal plastic bag to determine the contents. Boxes containing:

- ash bolts with immature larval parasitoids can be held in closed bags and boxes.
- immature *Oobius* on paper inside small plastic vials with screening, will require you to open the box and the plastic bag, and keep in a well-lit, air-conditioned room.
- adult parasitoids inside clear plastic shipment cups, will require your care to survive beyond the day of arrival
- To care for adult larval parasitoids (*Tetrastichus* and *Spathius*), open the box, remove and open the bags. Inside each bag will be a number of labeled cups containing small groups of live parasitoids. To maintain sufficient ambient moisture for the parasitoids, we recommend placing the rearing cups in a clear plastic storage tub with moistened paper toweling. Before placing the cups in the plastic tub, check each cup for the presence of honey. Honey provides the parasitoids with food and some moisture during shipping. If no honey is visible on the screening on the lids of the cups with *Spathius* or *Tetrastichus*, put two or three drops of honey on the screening and gently smear it.
- *Oobius* adults shipped in clear plastic cups do not require additional honey and should not be opened. The cups should be held in open coolers in a well-lit air conditioned room, as described above for the immature *Oobius* on paper.

TRANSPORTING PARASITOIDS TO FIELD SITES

Carry the cups or infested logs inside the boxes when transporting parasitoids to the field for release. For delayed releases they do not need to be re-bagged for local transport. Care should be taken to keep the box out of direct sunlight or other potentially hot (e.g., a sealed vehicle) environments. The trunk of a vehicle will suffice, but an air conditioned interior is even better, provided the vehicle will not be allowed to sit unattended in the sun for any period. ***Keep the box in the shade at all times*** because parasitoids are extremely sensitive to overheating. Keep the box closed except to remove the cups or logs with parasitoids for release. Carry the box carefully and avoid sudden movements. Parasitoids are extremely small and susceptible to drowning in droplets of water or honey if the cup is inadvertently shaken or dropped.

RELEASE OF PARASITOIDS

Adult Parasitoids. If possible, release the parasitoids in the morning or evening so they can move about in the environment before the onset of high afternoon temperatures. Carefully remove the snap-top lid with the tissue-paper liner when opening cups containing *Oobius* adults. Place the cup or vial and tissue next to the trunk of the tree. 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 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 throughout the release site.

Larval 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 pound into the tree trunk or a zip-tie or wire to hang the log from a branch. 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 wire, wrapped around a horizontal branch of the tree – this is particularly effective on large trees where trunk diameter makes it difficult to hang the log on the side of the trunk. Do not hang more than one log per tree; the parasitoids will establish better if they are spread out. The logs should remain in the field for at least 6 weeks to assure that all the parasitoids have emerged as adults. Remove the nails from the trees when you recover the logs because nails will harm sawmill equipment if the trees are harvested.

Oobius Parasitoid Pupae inside EAB Eggs on Paper in Small Plastic Cups (oobinators). Hang one oobinator per ash tree, and distribute them widely throughout release sites to encourage the spread and dispersal *Oobius*. Ash trees with flaky bark are preferred because EAB prefers to lay eggs on ash trees with flaky bark; these are often the larger diameter trees in the stand. Each oobinator must be left on the trees for at least 6 weeks for complete adult emergence. **Note: If the oobinators in your *Oobius* shipment have caps, do not forget to remove the caps before hanging them in the field.**

ENTER RELEASE DATA

Every time you release parasitoids, enter the following information into your GPS device or iPhone app and synchronize or enter directly into mapbiocontrol.org:

- **Release Date**
- **Release time**
- **Weather Conditions** (Sunny, Partly Cloudy, Foggy, Light Rain, Moderate Rain, Heavy Rain, Thunderstorms)
- **Wind Speed** (Light, Moderate, Strong)
- **Temperature** (Degrees Fahrenheit)
- **Number Female *Oobius* Released** (when releasing as pupae this will be an estimate)
- **Stage *Oobius* released** (Adult, pupae, both)
- **Number Female *Spathius* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Spathius* released** (Adult, pupae both)
- **Number Female *Tetrastichus* Released** (when releasing as pupae in ash logs this will be an estimate)
- **Stage *Tetrastichus* Released** (Adult, pupae, both)
- **Number Females** of other species released
- **Stage Other Released** (Adult, pupae, both)
- **Notes**

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. Yellow pan traps are inexpensive and easy to sample, but they do not give any indication of the number of EAB attacked by parasitoids. Collecting EAB eggs and larvae from trees allow certainty that the parasitoids are attacking EAB. However, these methods are more time consuming, and in order to recover larval parasitoids a tree must be cut down and debarked.

At least two consecutive years of recovery is required at each release site that is being sampled. You only need to sample one release site per county, although more is always better. All sampling to recover parasitoids should begin in the spring following the final release at a given site. Below we describe the four 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 can develop inside their host. *Tetrastichus* may have three to four generations per year. An EAB larva parasitized by *Tetrastichus* may 1) look healthy; 2) appear lumpy like a "braided rope"; 3) be replaced by a mass of small grub-like larvae (white), pupae (color ranges white to bluish-black) and/or adults (dark metallic blue); or, 4) 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* larva before pupation is complete) (Appendix B). The parasitoids pupate in the EAB gallery and may be recovered by debarking ash trees. Adults can be recovered in yellow pan traps during spring, summer, and fall, although they are most abundant in late summer in northern areas.

Spathius agrili* and *Spathius galinae are gregarious ectoparasitoids (external parasitoids) of EAB larvae, and all life stages live on the outside of the host. *Spathius* eggs and small 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 are fairly easy to see in the EAB galleries (Appendix B). *Spathius* requires a period of chill to break diapause, thus cutting trees for debarking should not take place before January. Like *Tetrastichus*, adults of *S. agrili* can also be recovered in yellow pan traps, and we assume this is also true for *S. galinae*.

Oobius agrili spends the winter in diapause inside EAB eggs, which are difficult to find sheltered between layers of bark and in bark crevices. EAB eggs are light brown or gold, whereas *Oobius*-parasitized eggs are often dark brown or black in color (Appendix B). Ash bark with EAB eggs can be scraped off trees in the field without injuring the tree, and the bark is dried and sifted to recover EAB eggs in the laboratory. Although *Oobius* adults are small, they can also be recovered in yellow pan traps.

NOTE: If you would like examples of parasitoid adults, larvae, pupae or cocoons to help with field identification, please contact the Rearing Facility 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

Felling trees to determine parasitoid establishment can be done in the fall, winter, or early spring when parasitoid densities are highest, at least one year after the final release at a given site. Select four trees near release trees that are alive (based on bark peeling and confirmation of live phloem), show signs of damage due to EAB (woodpecker holes, bark splits, epicormic shoots) and are less than 10-inches DBH. Give each tree a unique ID

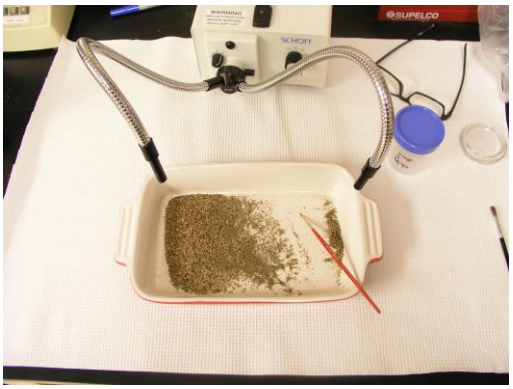
number, and record its DBH, location (GPS coordinates), and the date the tree was felled. If you have a handheld GPS unit, you can enter these data in the field before felling the tree.

DEBARKING

Before debarking the logs to recover larval parasitoids, scrape the bark to collect EAB eggs if bark samples were not previously collected in the field.

Recovering the Egg Parasitoid Oobius from Outer Bark Samples: Take bark samples from living, EAB-infested ash trees in the vicinity of the original release site in the field, or from trees felled for larval sampling. To sample live standing trees in the field, mark off a vertical area of bark 10 x 50 cm (or 10 x 100 cm if EAB density is low) on the south, southwest, or west side on the lower trunk (about 1-m above ground) on at least 10 trees. To collect the bark samples, tightly wrap a piece of heavy plastic sheeting around the base of the tree with duct tape Hold the edges of the sheeting up in the shape of an inverted cone (this method requires two people). Using a drawknife, shear off a thin layer of outer bark within the delineated area, and the bark debris will fall into the inverted plastic cone. Remove the duct tape and using the plastic sheeting, funnel the bark sample into a labelled large paper lunch bag, and return it to the laboratory.

Dry the sample at least one month at room temperature, place small aliquots of bark in a No. 14 covered soil sieve, and shake vigorously for several minutes to sieve EAB eggs, small insects and other small debris from the bark sample into a white ceramic backing pan. Using a dissecting microscope or magnifying glasses, sort the EAB eggs and small adult parasitoids from the debris. Send EAB eggs and adult wasps or high quality photographs to Leah Bauer (see cover page for phone number and email address) for identification.



Bark samples to recover eggs can also be taken from ash trees felled for other recovery work. We suggest taking one bark sample per tree from flaky bark from a log cut from the lower two meters of each tree. Measure the diameter of the log and determine the length of log necessary to sample a total surface area of 800 cm^2. For example, if the diameter (D) of the log is 8 cm, you would need to sample $800/(\pi*D) = 32$ cm. Shear off a thin layer of bark that is 32-cm wide around the circumference of the log.

***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 (with Anchorseal® for example) 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 friction-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 Juli Gould for identification (see page 38 for address). 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. The identifier will enter the final identification data into MapBioControl.

YELLOW PAN TRAPS

Many adult bee and wasp species are attracted to the color yellow. Parasitoid recovery studies have shown that yellow pan traps (YPTs) were effective at trapping the two larval parasitoids *Tetrastichus planipennisi* and *Spathius agrili*, and the egg parasitoid *Oobius agrili*. Other known EAB larval parasitoids (e.g. *Atanycolus*, native *Spathius* spp.,

Phasgonophora sulcata, *Balcha indica*) are also trapped, along with many other species of bees, wasps, flies, 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 09; manufacturer: Festive Occasion, East Providence, RI 01916)
2. one 6-inch right-angled shelf-bracket
3. three 1.25-inch long wood screws
4. one small binder clip for securing the collection bowl to the holding bowl.
5. weather-proof marking pen (e.g. Sharpie) and grease pencil (needed if bowls are wet)
6. three 6-inch zip-ties
7. 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.
8. rechargeable portable electric screw-driver with bit and extra battery pack
9. 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 (~2.5-cm square) in the bottom with a utility knife.

J. Hansen

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. organza) over the drainage holes to prevent loss of specimens during overflow. After the bracket and holding-bowl are mounted on the tree, set the 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 3, or 4. Do not put the traps on dead or very healthy ash trees. Place the YPT's on live-EAB infested ash trees throughout the release areas. If possible place the traps on or near the release trees.

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

When to deploy YPTs in the field? Deploy YPTs at EAB biocontrol release sites at least one year after the final parasitoid releases. All four parasitoids have multiple generations per year and adult parasitoids fly during the spring, summer, and early fall. The timing of possible captures in YPTs will depends on the climate in your area and the adult flight period of each species. If resources permit intensive sampling, we recommend deploying the YPTs when 300 Growing Degree Days have been accumulated, then throughout the spring, summer, and early fall. This will require visiting sites weekly, collecting the samples, and replacing the liquid in the bowl for the next week of sampling. If resources do not permit such intensive sampling, then we recommend sampling once per month through 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. 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 or two days. If shipment is delayed, store the samples in the freezer.

What do I do with these samples? If you have staff that can be trained to identify the three EAB parasitoids, then pre-screening can be done locally and suspect insects can be sent for positive identification. There are native insects in the genera *Spathius, Tetrastichus,* and *Oobius* that can be mistaken for the exotic species. If you choose to pre-screen the samples, please contact the rearing facility for examples of adult males and females of each species. Send samples by overnight shipping to Juli Gould, 1398 West Truck Road, Buzzards Bay, MA 02542. Enter recovery data into MapBioControl.

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 or using the iPhone/iPad app. 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. If you are entering data from multiple trees or yellow pan traps and you did not recover any suspect parasitoids, a specific ID is not required. Nonetheless you will need to make up an ID number because this field is mandatory.
- **Latitude** (dd.ddddd)
- **Longitude** (dd.dddd)
- **Site ID** Once you type in the Latitude and Longitude of your sample, the database will select some nearby sites from which to choose. Select the appropriate site. If you happened to find parasitoids not connected with any particular release or control site, simply select NO Site.
- **State**
- **Date Sample Collected**
- **Sample Method** (Yellow Pan Trap, Tree Debarking, Logs in Tubes, Bark in Tubes, Sentinel Eggs, Sentinel Larvae, Egg Collection, Other)

- **Number of Samples**
- **Possible EAB Parasitoids Recovered?** Yes or No

If you did recover some possible EAB parasitoids, record the date they were shipped for identification and the person they were shipped to. The data on the number of released parasitoids recovered will be entered into the database by the 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 the time and resources it will greatly assist researchers in determining which types of forest compositions are more likely to promote establishment of EAB parasitoids.

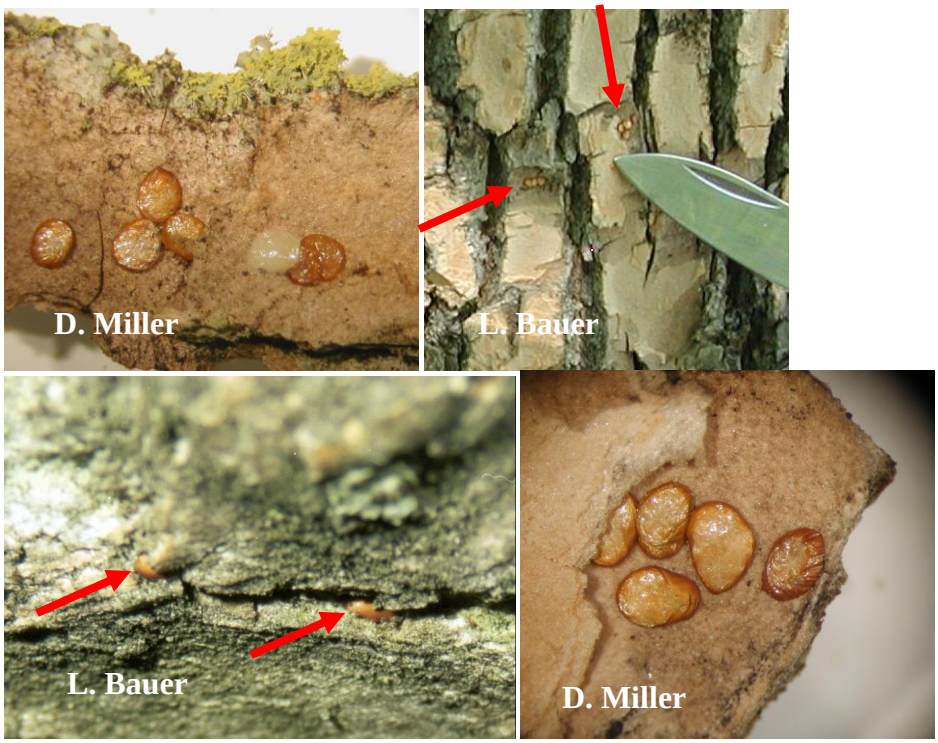
Ash Health Assessment

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

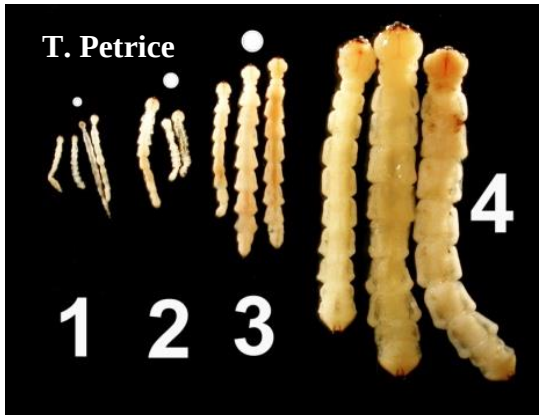
Mention of companies or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data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

EAB Life-Stages

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

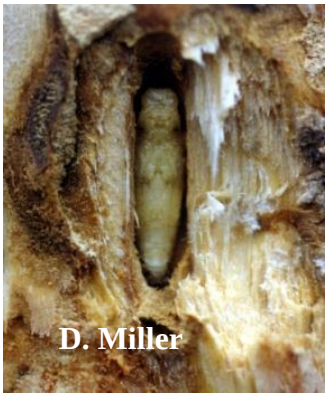


EAB larvae (1-4 indicates the four instars)



EAB larva

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 of three overwintering chambers, each with 2 holes filled with frass.

EAB Adult

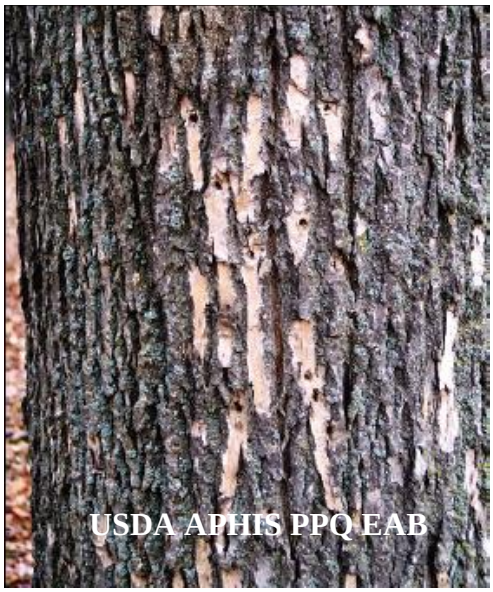
Signs of EAB infestation

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

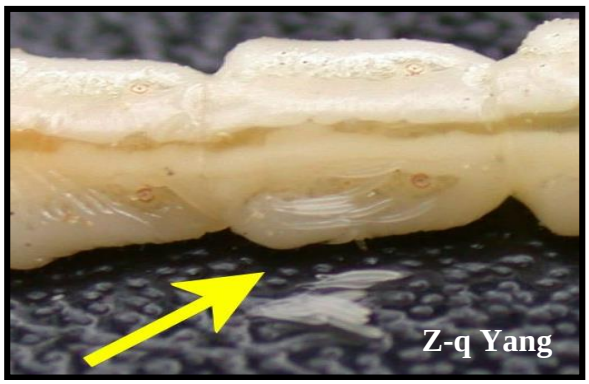
Additional photos and specific morphological and physiological information can be found in the EAB Program Manual at:

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

Life stages of EAB Parasitoids

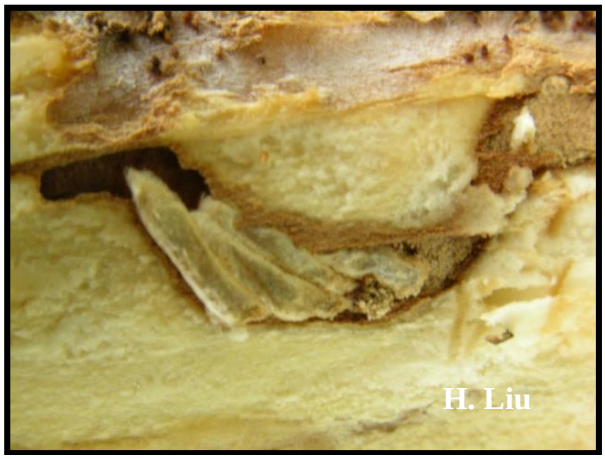
Spathius agrili

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.

Spathius galinae

Spathius galinae larvae and cocoons

Spathius galinae adult female

Tetrastichus planipennisi

Immature T. planipennisi larvae inside EAB

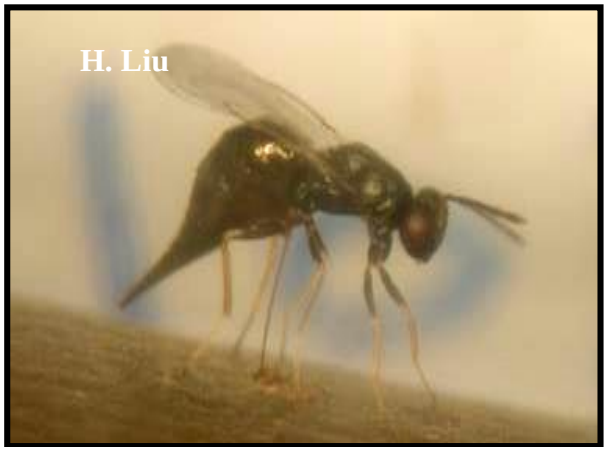
Mature *T. planipennisi* larvae inside an EAB larva

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

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



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 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.

Appendix D – Helpful Links

mapBioControl (to enter release and recovery 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://uspest.org/US/

General EAB Information

EAB Program Manual

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

APHIS Emerald Ash Borer Home Page

http://www.aphis.usda.gov/plant_health/plant_pest_info/
Click on "Pests and Disease Programs" then "Emerald ash Borer"

Emerald Ash Borer Info

http://www.emeraldashborer.info/
http://hungrypests.com/the-threat/emerald-ash-borer.php

Forest Service EAB Information

http://na.fs.fed.us/fhp/eab/

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