METHODS FOR STUDYING EMERALD ASH BORER PARASITOIDS IN THE FIELD

Leah Bauer,[1,2] Jian Duan,[3] Juli Gould,[4] Kristopher Abell[2,5] Deborah Miller,[1] Jason Hansen,[2] and Roy Van Driesche[5]

[1]USDA Forest Service (FS), Northern Research Station, E. Lansing, MI 48823
lbauer@fs.fed.us

[2]Department of Entomology, Michigan State University, E. Lansing, MI 48824

[3]USDA ARS, Beneficial Insects Introduction Research Unit, Newark DE 45433

[4]USDA-APHIS-PPQ CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542

[5]Department of Plant, Soil and Insect Science, University of Massachusetts, Amherst, MA 01003

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), is an invasive phloem-feeding beetle from Asia that attacks ash (*Fraxinus* spp.) trees. EAB was determined to be the cause of extensive ash tree mortality throughout southeast Michigan and nearby Ontario in 2002. For several years, regulatory agencies attempted eradication of EAB, but these efforts were unsuccessful and were later abandoned in favor of management.

Classical biological control is considered the only sustainable method for long-term management of EAB in forested ecosystems. In areas of China where EAB is native, three hymenopteran parasitoid species were discovered parasitizing EAB eggs [*Oobius agrili* (Encyrtidae)] or larvae [*Tetrastichus planipennisi* (Eulophidae) and *Spathius agrili* (Braconidae)]. After completing studies on EAB population dynamics in China, parasitoid biology and host ranges, and preparation of an Environmental Assessment, USDA APHIS issued permits for releases of these parasitoid species in Michigan in 2007. Parasitoid releases were expanded to include study sites in Ohio and Indiana in 2008, and Illinois and Maryland in 2009. In 2010, an APHIS EAB-parasitoid rearing facility in Brighton, Michigan became operational, and parasitoid releases expanded to most of the EAB-infested states in 2011.

Until recently, determination of parasitoid overwintering or establishment in the field involved destructive sampling of EAB-infested ash trees. Using this method, one or more parasitoid species have been confirmed as established in Michigan, Ohio, Maryland, Indiana, and Illinois. Because ash trees are increasingly scarce at our field sites, we developed alternate methods for detecting these introduced parasitoids.

Methods for recovery of the egg parasitoid *O. agrili*: The usual methods to detect overwintering or establishment of *O. agrili* at field sites are to 1) search bark for EAB eggs and rear out egg parasitoids in the laboratory (see symptoms of egg parasitism below) or 2) to fell EAB-infested ash trees at or near the original release epicenter in late winter or early spring and hold logs or bark for rearing. Logs or bark samples are then placed in dark cardboard-rearing tubes at room temperature

for 6 to 8 weeks and emerging insects are collected every few days from a clear plastic emergence cup attached to the end of each tube and held for identification. Logs and bark samples (stored in paper bags) can be refrigerated (4°C) for up to three months. Although *O. agrili* successfully emerge from ash log and bark samples, this method requires a large amount of laboratory space and does not provide data on parasitoid prevalence as the number of EAB eggs in the sample is unknown.

An alternate method for recovering *O. agrili* in the field includes hanging small ash logs with EAB eggs (egg-sentinel logs) on ash trees. Egg-sentinel logs (ESLs) were made in the laboratory by exposing small ash logs (~5-cm diameter × 25-cm long) to gravid EAB females and fresh ash foliage in 3.8-L ventilated plastic jars until $\geq$50 eggs were laid on each log (2 to 3 days). Before exposure, the logs' ends were dipped in paraffin and each log was wrapped with a spiral of curling ribbon to stimulate EAB to lay eggs beneath the ribbon. After the eggs were counted and marked, the ribbon was placed back over the eggs, and ESLs were hung on ash trees in the field for one to two weeks. To determine percent egg parasitism, ESLs were returned to the laboratory and the eggs on the log observed under a dissecting microscope for signs and symptoms of egg parasitism. These signs include 1) emergence of *O. agrili* from EAB eggs when held in a vial or Petri dish; 2) circular emergence-holes on exposed surfaces of eggs from which adult wasps emerged earlier; 3) dark coloration of EAB eggs; or 4) presence of *O. agrili* life stages inside eggs when dissected. The prevalence of egg parasitism in collections of naturally occurring eggs tends to be underestimated because EAB-egg shells remain on ash bark for several years. On the other hand, natural egg parasitism rates on ESLs may be overestimated if the density of the eggs offered on the log is too high. Thus, deployment of more ESLs with fewer eggs/ESL on more trees may better estimate the prevalence of *O. agrili* in the field.

ESLs are useful for detecting changes in *O. agrili* parasitism over time and space. For example, at one Michigan biological control release plot where *O. agrili* adults had previously been released over a three-year period (2007-2009), we deployed ESLs from on each of five or six ash trees in the last three years. We found 3.9% egg parasitism on ESLs deployed in 2009, 6.1% on ESLs deployed in 2010, and 20.4% in 2011. Moreover, in 2011 we made the first detection of *O. agrili* parasitism in a control plot (0.2% egg parasitism) using ESLs placed ~800 m away from the point of initial release. In 2011, we also used ESLs to assess the phenology of *O. agrili* at this release plot over the course of the full season (see Abell et al., in these proceedings).

Methods for recovery of the larval parasitoids *T. planipennisi and S. agrili*: The usual method to recover EAB larval parasitoids in the field is destructive sampling of infested ash trees. The sampled trees are debarked in the field or laboratory. Immature EAB and associated parasitoids are extracted and reared in the laboratory to obtain adults for identification or dissected to obtain endoparasitoids. Another option is to return ash logs to the laboratory and hold them in cardboard-rearing tubes at room temperature (~25°C) and wait for parasitoids to emerge. With the latter method, however, the parasitoid host species is unknown.

Detection of larval parasitoids in the field may also be achieved by hanging small ash logs containing EAB larvae (larval-sentinel logs) on ash trees. Larval-sentinel logs (LSLs) were made by cutting small ash logs (~5 cm dia × 18 cm long), inserting five 3^{rd}- or 4^{th}-instar EAB larvae in chambers cut under bark flaps, sealing the ends of the logs with Parafilm, and hanging them on ash trees for one week. The LSLs were returned to the laboratory, the parasitoids removed and reared to the adult stage for identification. In 2010 and 2011, we used LSLs to detect parasitism by *T. planipennisi* at three sites where this species had been released over a two year period (2008-2009). In 2010, EAB-larval parasitism rates in the LSLs by *T. planipennisi* were 7%, 45%, and 23% at the three sites. At these same sites in 2011, parasitism rates by *T. planipennisi* in sentinel logs were 3%, 22%, and 32%, respectively. Interestingly, at the two sites where *T. planipennisi* parasitism of EAB larvae declined in 2011, parasitism by native parasitoids in the genus *Atanycolus*, exceeded 50%. Although *S. agrili* was also released at these sites, it was not detected using the LSLs.

We also tested the use of yellow pan traps (YPTs), which are known to be attractive to certain Hymenoptera, including some parasitoid species. YPTs were made from yellow plastic disposable bowls (~12 cm dia) containing soapy water or non-toxic antifreeze. These bowls were mounted horizontally on ash trees using shelf brackets. After three to five days, the contents of these traps were collected, returned to the laboratory, stored in ethanol, and EAB parasitoids removed under a dissecting microscope. Of the introduced EAB biocontrol agents, *T. planipennisi* was more commonly captured by this trap than *S. agrili*; however, no *O. agrili* were recovered using this method. YPTs are a simple method to detect establishment of larval parasitoids at release sites. To optimize parasitoid catch, however, we recommend YPTs be placed 1) at sites no sooner than two years after the last parasitoid release; 2) near the original point of release; and 3) during late summer or early fall when EAB parasitoid population densities are high.

Summary. At our EAB biological study sites in central Lower Michigan, most of the larger ash trees died off within one or two years of initiation of our studies. Survival and continued growth younger ash trees in the stands provide us with host material to destructively sample. The numbers of such trees in and around our field sites, however, are limited due to limited recruitment of ash seedling into the stands. Loss of ash trees at our study sites will adversely affect parasitoid populations and the results of our research. We have determined that larval- and egg-sentinel logs and yellow pan traps are useful for determining the presence or absence of EAB parasitoids in the field.