Chapter 7: Trapping Techniques for Emerald Ash Borer and Its Introduced Parasitoids

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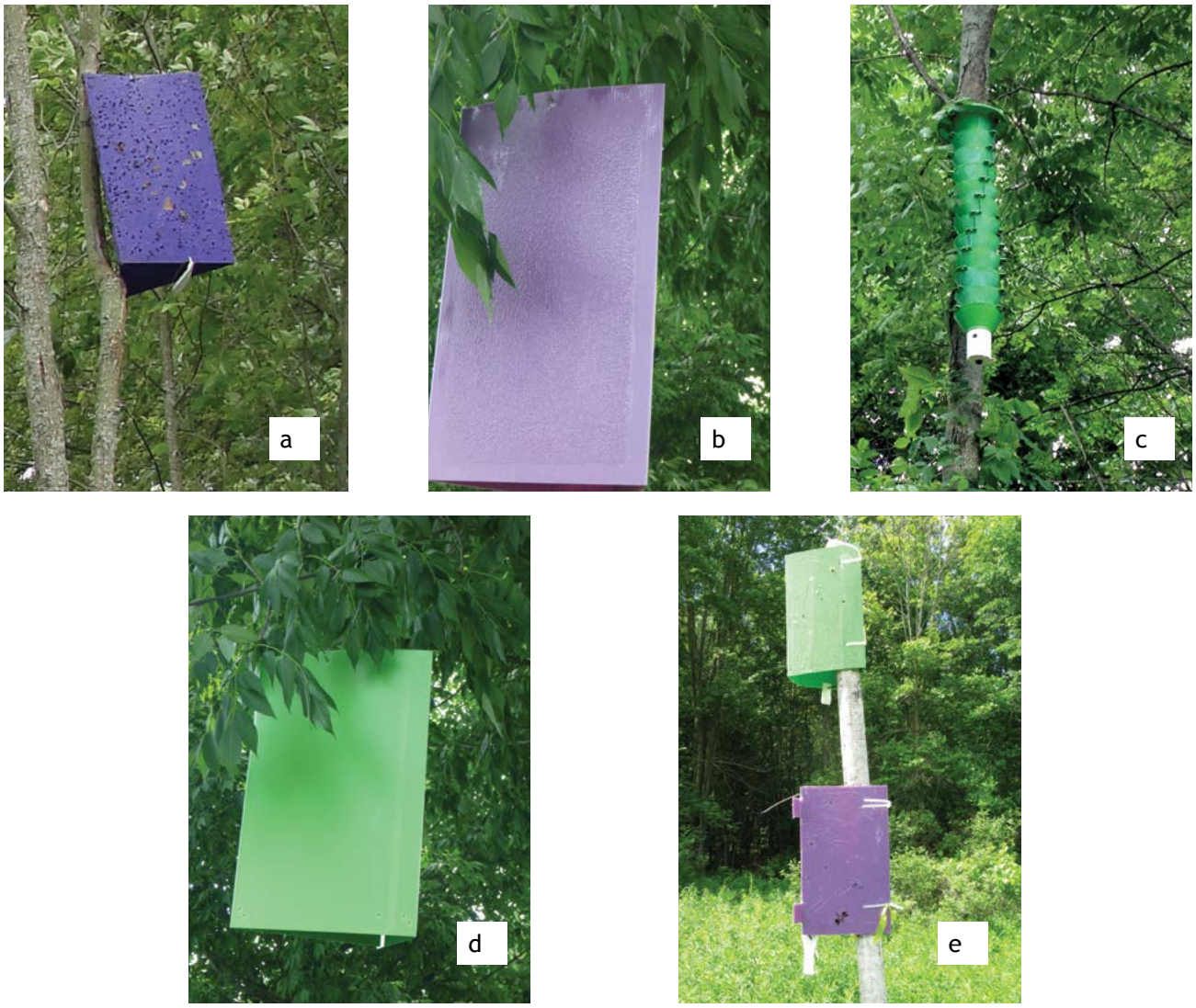
SURVEY AND DETECTION OF EMERALD ASH BORER

As soon as emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) was discovered near Detroit, Michigan, USA, in 2002, surveys were initiated to delimit the extent of the infested area. These initial delimitation surveys were based on visual assessments using external symptoms because at the time no other detection tools were available and nothing was known about EAB responses to chemical or visual stimuli. Surveys were supplemented by tracing movement of nursery stock shipped from Detroit to other locations to detect new infestations of EAB. External symptoms of EAB infestation, which include D-shaped exit holes, dieback and crown thinning, epicormic shoots, and bark splits over galleries, are not apparent until one or more years after trees are infested by which time some adult beetle emergence may have occurred, allowing dispersal to other locations (Poland and McCullough, 2006). Therefore, visual surveys that rely on detecting infested trees are not effective for discovery of low-density infestations.

As of 2014, development of better detection tools for EAB remained an important need for the regulatory program. Research on EAB behavior demonstrated that adult beetles respond to volatiles emitted by stressed ash (Rodriguez-Saona et al., 2006) and preferentially oviposit on girdled trees (McCullough et al., 2009a,b). Based on this finding, in 2004 the Michigan Department of Agriculture implemented a statewide survey employing grids of girdled trap trees (Rauscher, 2006; Hunt, 2007). Large, open-grown ash trees were girdled in spring before EAB emergence by removing a band of bark and phloem, approximately 16 cm wide, around the whole circumference of the tree. A band of plastic wrap, approximately 30 cm wide, was placed on the trunk above the girdle and coated with Tanglefoot insect trapping glue. Girdled trap trees were visually inspected during the summer to detect EAB adults on sticky bands; in fall or winter, girdled trees were felled and sections of the log were peeled to locate EAB larvae or galleries. Grids of over 10,000 trap trees were used for detection surveys in Michigan and several surrounding states up through 2008. While girdled trees are the most effective tool for detecting EAB (McCullough et al., 2011; Mercader et al., 2013), debarking trees to locate larval galleries is costly and labor-intensive, and suitable trees are not always available. Consequently, emphasis was placed on development of traps and lures that incorporated visual or olfactory cues to attract and capture EAB adults.

Odors from the leaves of stressed ash trees (Rodriguez-Saona et al., 2006), green leaf volatiles, especially *cis*-3-hexenol (de Groot et al., 2008; Grant et al., 2010, 2011; Poland et al., 2011), and sesquiterpene volatiles from ash bark elicit antennal responses and are attractive to EAB. Many of these attractive compounds are present in a natural tree oil called Manuka oil (Crook et al., 2008), and for this reason this oil was often incorporated into EAB traps.

Male and female EAB are sensitive to light in the ultraviolet (UV), violet, and green (420-430, 460, and 530-560 nm, respectively) ranges of the electromagnetic spectrum, while mated females are also sensitive to light in the red (640-670 nm) range (Crook et al., 2009, 2012). The beetles are attracted to green or purple traps hung in both the open and the ash canopy (Crook et al., 2009; Francese et al., 2010).



Figures 1 a-d. Various trap designs, colors and lure combinations suspended in the canopy of an ash tree: .(a) Dark purple sticky prism trap. (Photo credit: Therese Poland); (b) Light sabic purple sticky prism trap. Photo credit: Therese Poland); (c) Green multiple funnel trap coated with Fluon. (Photo credit: Toby Petrice); (d) Green sticky prism trap. (Photo credit: Therese Poland); (e) Green and purple double decker trap. (Photo credit: Therese Poland)

Males, which tend to hover near the canopy of ash trees (Rodriguez-Saona et al., 2007), are captured in higher proportions in green traps hung in the canopy of ash trees and baited with green leaf volatiles; in contrast, females, that oviposit on the trunks of ash trees are captured in higher proportions in purple traps hung below the canopy and baited with bark sesquiterpenes (Crook and Mastro, 2010; Grant et al., 2011). There is also evidence that close range or contact pheromones are involved in mate recognition and mating behavior (Lelito et al., 2009; Pureswaran and Poland, 2009) and that a female-produced volatile pheromone, *cis*-lactone, increases attraction of males to green canopy traps baited with green leaf volatiles (Silk et al., 2009, 2011; Ryall et al., 2012).

Artificial traps were first used by USDA Animal Plant Health Inspection Service (APHIS) in a national EAB detection survey in 2008 (Crook and Mastro, 2010). Traps consisted of 3-sided prisms made of standard dark purple corrugated plastic (Coroplast Inc., Dallas, TX; 421 nm, 16.3% reflectance; 605 nm, 9.5%; 650 nm, 14.2%). Traps were coated with clear insect trapping glue, hung in the canopy of ash trees, and baited with Manuka oil lures with release rates of 50 mg/day (Synergy Semiochemicals, Burnaby, B.C.) (Fig. 1a). Various trap designs, colors, and lure

combinations were tested and detection surveys modified to incorporate the latest research findings. Starting in 2014, a new lighter shade of purple (Great Lakes IPM, Vestaburg, MI; Sabic purple, 413 nm, 32.8%; 613 nm, 18.8%; 650 nm, 28.5%) was employed for the sticky prism traps hung in the canopy of ash trees. Also, cis-3-hexenol lures releasing 50 mg/day (Scentry Biologicals, Inc., Billings, MT) have been added to the Manuka oil lures (USDA APHIS, 2014) (Fig. 1b).

Other promising traps under evaluation as of 2014 included (1) green (530 nm, 57% reflectance) multiple funnel traps (Chemtica Internacional, San Jose, Costa Rica) coated with Fluon, a slippery polymer (Northern Specialty Chemicals, Dudley, MA), and baited with cis-3-hexenol released at 50 mg/day (Scentry Biologicals, Inc., Billings, MT) (Francese et al., 2011) (Fig. 1c), (2) green (540 nm, 49% reflectance) sticky prism traps hung in the canopy of ash trees baited with cis-3-hexenol and the EAB pheromone cis-lactone (Sylvar Technologies, Inc., Fredericton, NB) (Ryall et al., 2012) (Fig. 1d), and (3) double decker traps made of a 10 foot PVC pole to which a green sticky prism (540 nm, 49% reflectance) is attached at the top and a light purple sticky prism (413 nm, 32.8%; 613 nm, 18.8%; 650 nm, 28.5%) is attached 60 cm below (Great Lakes IPM, Vestaburg, Michigan); both prisms are baited with two cis-3-hexenol bubble caps releasing 3.7 mg/day per bubble cap (ConTech Enterprises, Inc., Delta, B.C) (Poland et al., 2011, Poland and McCullough, 2014) (Fig. 1e).

The 2014 national emerald ash borer survey included (1) a nationwide survey of 8800 traps, set outside the 100 mile wide buffer zone surrounding the known infested area in locations at risk for introduction and establishment of EAB and (2) a leading edge survey employing 13,200 traps set within the 100 mile wide buffer zone. Traps were set within 1 km^2 cells that were selected using a risk-based model that incorporated risk factors that included proximity to campgrounds, major transportation arteries, truck stops, sawmills, firewood vendors, tree nurseries, recently landscaped properties, and high attendance cultural event sites. A trap was placed within each 1 km^2 cell, avoiding habitats of threatened or endangered species. Traps were placed in the lower to mid-canopy of ash trees, preferably 20 cm or more in diameter, along edges or open areas on the sunny side of trees. The bottom edge of the trap was 150 cm or more above ground (USDA APHIS, 2014). In addition, any ash trees within each 1 km^2 cell exhibiting two or more symptoms of emerald ash borer infestation (dieback, epicormic shoots, bark splits, woodpecker damage, D-shaped exit holes, or visible serpentine galleries) were destructively sampled by removing bark to reveal emerald ash borer galleries and larvae.

Traps were placed in the field just before 250 growing degree days (base 10 °C) were accumulated, which corresponds approximately to the time when emerald ash borer emergence begins. Lures were replaced within 60 days. Traps were checked at a minimum when lures were replaced and when traps were taken down. All captured EAB and suspect beetles were collected and submitted to the State Plant Health Director or APHIS representative for species determination. Traps remained in place until after August 1 and 833 growing degree days (base 10 °C) had accumulated (USDA APHIS, 2014).

SURVEY AND DETECTION OF INTRODUCED EAB PARASITOIDS

For parasitoids introduced for biological control, both their establishment and impact on the target pest must be measured. Establishment means the development of a successfully reproducing, self-sustaining population of the natural enemy, complete with overwintering survival for one or more years. Parasitoid establishment cannot be determined until at least one year after parasitoid release. Evaluating the impact of a natural enemy on the population of the target pest requires an estimate of the mortality caused by the natural enemy to the host; often this is equivalent to the generational rate of percentage parasitism in the naturally occurring host population in the field. Some parasitoids can kill hosts by means other than parasitoid reproduction, such as host feeding or stinging hosts without laying any eggs (DeBach, 1943; Van Driesche and Taub, 1983; Jervis and Kidd, 1986; Kidd and Jervis, 1989; Heimpel and Collier, 1996; Jervis et al., 1996); however, the

Figure 2 a,b. Laboratory produced emerald ash borer eggs on bark flakes placed under bark flaps cut into ash trees in the field. (Photo credit: Jian Duan)

parasitoids currently being released against emerald ash borer do not have these behaviors.

In the case of the emerald ash borer, there are two beetle life stages targeted by parasitoids: the egg and larva. The egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al., 2005) and two larval parasitoids, *Tetrastichus planipennis* Yang (Eulophidae) (Yang et al., 2006) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al., 2005), have been imported and released in North America (Bauer et al., 2008). Various methods have been developed to assess the establishment and impact of these parasitoids and they are reviewed in this chapter.

EGG PARASITOIDS

Three approaches have been used to detect establishment or measure the impact of the egg parasitoid *O. agrili*: (1) deploying laboratory-produced host eggs in the field as sentinel eggs, (2) using yellow pan traps to passively collect *O. agrili* adults, and (3) collecting wild (naturally occurring) emerald ash borer eggs in the field.

Sentinel Eggs

Deployment of sentinel eggs can detect the presence of *O. agrili* at particular sites, which, if appropriately timed, can indicate establishment. Several methods have been developed for field-deployment of emerald ash borer eggs produced in the laboratory. The first of these involves cutting a small flap of bark on an ash tree and placing EAB eggs under this flap (Fig. 2). Eggs can be collected later and examined for parasitism. This method, however, suffers from a high degree of egg predation (Duan et al., 2011). The second method is based on the field-deployment of ash logs bearing laboratory-laid EAB eggs. These egg sentinel logs (ESL) are made by wrapping curling ribbon around a bolt of ash (ca 5 cm in dia by 25 cm long) and placing them in a container with gravid EAB for several days (Fig. 3). The tight space between the curling ribbon and the ash bolt stimulates EAB oviposition (Fig. 4) and partially conceals the egg from predators in the field. A more detailed description of ESL production can be found in Duan et al. (2012a) and USDA APHIS/ARS/FS (2013). Once produced, ESL units can be hung on or near ash trees and left for several weeks. It should be noted, however, that depending on temperature EAB eggs are only suitable for parasitism up to the development of the neonate host larva (approximately 8-10 days after oviposition). Once collected from the field, eggs on ESL units can be held in the laboratory to rear *O. agrili* adults. Alternatively, each egg can be inspected under a microscope for visual signs of parasitism (Fig. 5). A third method of deploying sentinel eggs is to place host eggs inside various protective enclosures, such as plastic cups (with or without a screened opening) or pouches made entirely of screening. Screening is used to exclude predators while allowing access to eggs by *O. agrili.* While field recoveries of *O. agrili* have been made using this method, it

Figure 3. Egg sentinel log with curling ribbon in container with emerald ash borer. (Photo credit: Deborah Miller)

Figure 4. Egg sentinel log with curling ribbon removed showing EAB eggs. Black eggs are parasitized and brown eggs are unparasitized. (Photo credt: Kristopher Abell)

Figure 5. (a) Parasitized EAB egg with parasitoid emergence hole and typical black coloration. (b) Parasitized EAB egg with meconium inside visible due to atypical brown coloration. (Photo credit: Deborah Miller)

generally seems less effective compared to use of ESL units. Currently, therefore, of the three methods used to deploy emerald ash borer eggs in the field to detect egg parasitism, use of sentinel egg logs is the preferred method (Fig. 6).

Yellow Pan Traps

Yellow pan traps are yellow plastic bowls (Fig. 7) mounted to ash trees with a shelf bracket nailed to the tree. A second bowl can be placed inside the mounted bowl to allow for easy removal and processing of the sample. This second bowl is filled with a 20% clear propylene glycol solution and a drop of unscented detergent. Yellow bowls are used because this color is generally attractive to many parasitoids, and detergent decreases the surface tension of the water, causing most insects to sink and drown. Trap contents

Figure 6. Egg sentinel log suspended from an ash tree in the field. (Photo credit: Kristopher Abell)

Figure 7. Yellow pan trap. (Photo credit: Leah Bauer)

Figure 8 a,b. Emerald ash borer eggs on ash bark in the field. (Photo credit: Jian Duan)

Figure 9. Removal and collection of outer ash bark with a drawknife for assessment of *Oobius agrili* using emergence tubes and bark sifting. (Photo credit: Kristopher Abell)

Figure 10. Emergence tubes used to collect emerging *Oobius agrili* from bark samples. (Photo credit: Deborah Miller)

should be collected after several days depending on temperature to avoid rotting. Once collected, the contents of the pan trap can be examined for the presence of *O. agrili* adults (JG, unpublished data). A detailed step-by-step guide to the construction and setup of yellow pan traps can be found in "Emerald Ash Borer Biological Control Release and Recovery Guidelines" (USDA APHIS/ARS/FS, 2013). Yellow pan traps are non-selective and may not detect low density populations of *O. agrili.*

Naturally Occurring EAB Eggs

Assessing the impact of *O. agrili* (i.e., percent parasitism for EAB populations) is a more difficult task than determining if *O. agrili* is established at a site. To assess the impact of *O. agrili* on naturally occurring EAB egg populations, EAB eggs must be collected in the field. Finding EAB eggs in the field is difficult because adults lay their eggs between layers of bark or in bark fissures on ash trees (Fig. 8). Two methods have been developed to collect wild EAB eggs. The first collection process is a timed visual inspection of ash bark, using a utility knife to parse away bark layers. An arbitrary but fixed amount of time (generally 30 minutes) is spent searching each tree to maintain consistent sampling effort. Eggs found are returned to the laboratory to be inspected with a dissecting microscope for signs of parasitism (Fig. 5) (Duan et al., 2011, 2012a).

A second method to measure rates of parasitism in wild EAB eggs is based on the physical removal of the outer bark of ash trees over a fixed area, inside of which layers of bark are scraped off using a drawknife (Fig. 9). If assessing establishment is the only goal, then sampled bark can be placed in emergence tubes (Fig. 10) and monitored for *O. agrili* emergence. Emergence tubes are typically made from cardboard mailing tubes, but other light-excluding containers can be used. One end of the tube is sealed against light while an inverted funnel and translucent collection cup (Fig. 11) is mounted on the other end. Tubes should be held in a well lit environment at 18-32 °C. At low densities, *O. agrili* may be difficult to detect because the parasitoids do not always find their way out of the emergence tube and into the collection cup. A more reliable way to detect *O. agrili* in bark

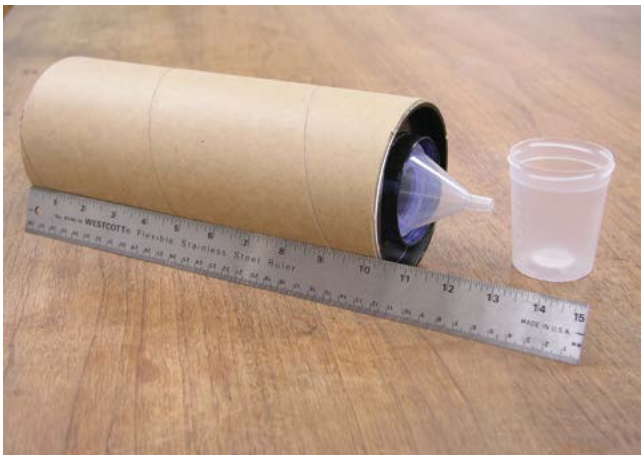


Figure 11. Close-up view of *Oobius agrili* emergence tube, cup and funnel. (Photo credit: Deborah Miller)

Figure 12. Sifting bark samples using standard nylon window screening. (Photo credit: Deborah Miller)

samples, which also allows for assessment of impact, is to examine the collected bark. While a complete search of the entire bark sample would be the most effective, it takes too long. Therefore, a subsampling approach was developed that involves sifting the bark sample with standard nylon window screening and determining rates of parasitism in eggs that pass through the screen. The bark sample is placed on window screening and shaken for three minutes (Fig. 12). Many eggs are dislodged while shaking and fall through the openings in the screening along with small bits of bark debris. The material that passes through the sieve is then examined for EAB eggs using a microscope, and each egg is evaluated for parasitism. An estimate of percent parasitism can be obtained from each of these methods (timed visual search and bark sifting) by dividing the number

of parasitized eggs by the total number of eggs (parasitized and not, both emerged and not emerged, live and dead).

Advantages and Disadvantages of Methods for Detection of Egg Parasitoids

The use of sentinel eggs, whether under bark flaps, on ESL units, or in protective enclosures, is subject to predation. Very often predators may remove most, or even all, sentinel eggs. The presence of curling ribbon on the logs used in the ESL units and screening over protective enclosures around the ESL unit both reduce but do not eliminate predation. Additionally, a substantial amount of infrastructure and manpower is needed to maintain an EAB colony, which is required to produce eggs for field deployment. When creating ESL units, a sufficient number of eggs (~50-100) must be produced per log and deployed in the field within 2-3 days. Older eggs are not preferred for parasitoid oviposition and their deployment produces little useful data. Time of deployment of sentinel eggs must also be carefully considered to coincide with seasonal occurrence of *O. agrili* adults. Deployment of sentinel eggs too early or too late in the year would result in false negatives when assessing establishment. In Michigan, *O. agrili* adult females first appear after approximately 445-556 degree days (base 10 °C) (Abell, unpublished data).

Yellow pan traps may be a relatively easy method to assess establishment of *O. agrili.* Unlike methods using sentinel eggs, pan traps do not require the maintenance of an EAB colony to produce eggs and the time constraints associated with egg viability are not an issue. Furthermore, since pan traps have the potential to also catch larval parasitoids of EAB this may increase their utility. However, there are several important disadvantages to consider. First, the incidental trap-catch of other similar-looking hymenoptera or other insects can be substantial. When such incidental catch is high, more time is required to examine and sort through the sample, which is particularly difficult considering the small size of *O. agrili.* Because of its small size, *O. agrili* can often become entangled in the setae of other insects making them easy to miss. Second, the effectiveness of yellow pan traps is largely unknown. Some work has shown pan traps to be more effective than ESL units and other sentinel egg methods, while other work has shown the opposite (Parisio, unpublished data; Bauer et al., 2011a).

Timed visual egg surveys and bark sifting allow for assessment of establishment and estimation of percent parasitism of naturally occurring field populations, but each has disadvantages to consider. Both methods collect EAB eggs from several generations and there is no way to differentiate old eggs from new ones. Because of this it can be difficult to assess year-to-year fluctuations in rates of parasitism at a site. Consideration of aspect (cardinal direction) of the sampling point on the tree is also important for each method. Sampling from only one side of a tree may introduce a bias. In general, sampling around the full circumference of the tree is recommended for estimating field rates of parasitism. However to only estimate establishment, egg density and parasitism rates are greatest on the south and west sides of tree (Abell et al., 2014). Visual egg surveys have several special disadvantages: finding eggs on standing ash trees in the field is difficult even with the aid of a magnifying lens, searching is affected by light and weather conditions in the field, and the process of removing small bits of bark while searching likely results in the loss of some eggs along with the removed bark. The bark sifting method does not have these disadvantages, but is more time consuming.

Regardless of the method used, *O. agrili* is particularly challenging to sample. Work done using all the above methods has shown that, at least during the first several years following the species release at a site, *O. agrili* has a very patchy distribution. Often, only 10-20% of trees sampled within several hundred meters of each other will result in recovery of *O. agrili* (Abell et al., 2011). Therefore a large number of trees (>10) need to be sampled to adequately assess *O. agrili* levels.

LARVAL PARASITOIDS

Several methods have been used to detect establishment and assess impact of larval parasitoids of emerald ash borer: (1) deployment of laboratory-reared EAB larvae in the field as sentinels, (2) using yellow pan traps to passively collect adult parasitoids,

and (3) collecting naturally occurring EAB larvae in the field for dissection or rearing.

Sentinel Larvae

There are several methods that employ sentinel larvae to assess establishment of parasitoids of emerald ash borer larvae. Larval sentinel logs (LSL) are similar in concept to the ESL units described previously for detection of the egg parasitoid. To construct LSL units, third to fourth instar EAB larvae are inserted into ash bolts approximately 5 cm in diameter and 25 cm long. Bolts are sealed on both ends with paraffin wax to prevent desiccation. To insert larvae, a portion of inner bark and wood approximately the same size as an EAB larva is excavated from the log, and an EAB larva is placed in the grove and covered by the remaining flap of outer bark (Fig. 13). Several EAB larvae can be inserted into a bolt in this manner. Then, after the outer bark flaps are secured, the area of the log where a larva has been inserted is further protected by wrapping it with parafilm. Care should be taken to sterilize the tools used to create excavations and handle larvae to avoid introducing pathogens. LSL units are then placed on ash trees in the field (Fig. 14) and left in place for 1-2 weeks. How long LSL units last in the field depends upon the age of EAB larvae and the temperature. Since EAB larvae tunnel into the heartwood of ash to pupate, they become inaccessible to parasitoids at that point. Temperature affects the rate of development of EAB larvae; also higher temperature increases desiccation of LSL units. LSL units can also be produced by placing emerald ash borer eggs on ash bolts and allowing newly hatched larvae to bore into bolts; bolts are then held at a constant temperature until larvae reach the appropriate instar. This second method, however, is less desirable because the number of EAB larvae in each bolt will be unknown since some eggs won't hatch and some larvae will die. In addition, LSL units produced in this manner seem to be less effective at detecting parasitism, possibly because cuts made when inserting larvae emit volatiles that attract parasitoids (Abell, unpub.). These two methods – inserting larvae or affixing eggs to bark – can also be applied to live ash trees in the field (Ulyshen et al., 2010; Abell et al., 2012). Additionally, adult EAB can be caged directly onto the trunk of live ash trees and allowed to oviposit eggs (Duan et al., 2014).

Figure 13. EAB larvae placed in excavated area of an ash bolt to create a larval sentinel log. (Photo credit: Kristopher Abell)

Figure 14. Larval sentinel log hung on an ash tree in the field. (Photo credit: Kristopher Abell)

Yellow Pan Traps

The setup, advantages, and disadvantages of pan traps to capture EAB larval parasitoids are much the same as when they are used to detect egg parasitoids, as described above. As stated above, yellow pan traps are non-selective and may not detect low-density

populations of released EAB larval parasitoids. Recently the pheromones of *Spathius agrili*, *Spathius floridanus* Ashmead, and *T. planipennisi* have been identified (Bauer, et al., 2011b, Cossé et al., 2012), and these materials can be used as attractants in combination with yellow pan traps to increase trapping efficiency.

The pheromones for the two *Spathius* species are male-produced aggregation pheromones attracting both male and female insects. The pheromone for *T. planipennisi* is a female-produced sex pheromone attracting males.

The attractiveness of synthetic *S. agrili* pheromone was tested in a large (3.7 x 6.1 x 3.7 m) outdoor field cage using eight (1.8 m high) evenly spaced potted evergreen ash (*Fraxinus uhdei* [Wenz.] Lingelsh.) plants. Yellow sticky board strips (Fig. 15) were placed in each plant halfway up. The pheromone was impregnated into rubber septa, affixed to the sticky boards. Approximately 45% of the released males and 50% of the released females were recaptured on the pheromone-baited traps during the 24 h trapping periods (Fig. 16) (Cossé et al., 2012), compared to 10% of released males and 5% of released females for yellow traps without pheromones. Field trapping of *S. agrili* using yellow pan traps and pheromone has not yet been demonstrated due to a lack of established populations of *S. agrili*.

For *T. planipennisi,* wind tunnel behavioral studies have demonstrated that male *T. planipennis* are highly sensitive to a female-produced pheromone with optimal responses to pheromone at 20 pg/µl. Under summer conditions, this dosage of pheromone is likely to be attractive for *T. planipennisi* males for at least two weeks. A field test was run in August-September, 2013 in East Lansing, Michigan where *T. planipennisi* has an established population. Twenty yellow pan traps were deployed following the method described, ten with and ten without pheromone lures. Septa were replaced by fresh ones after two weeks. Of 40 males trapped, 39 were captured by pheromone-supplemented traps, while control traps (yellow only) caught one parasitoid (Fig. 17).

The above results demonstrate that EAB parasitoid pheromones can increase efficiency of yellow pan traps. Pheromones of *Spathius* sp. and *T. planipennisi*

Figure 15. Field cage setup for release and recapture of male and female *Spathius agrili* with yellow sticky traps baited with parasitoid pheromone. (Photo credit: Allard Cossé)

are stable under field conditions and only small amounts of the pheromones are needed to attract the target parasitoids. A disadvantage of using pheromones is that they will have to be synthesized, since the compounds are not commercially available.

Naturally Occurring EAB Larvae

Sampling naturally occurring EAB larvae is the only way to estimate percent parasitism by larval parasitoids. To collect EAB larvae, the bark of living EAB infested ash trees is peeled off, usually with a drawknife (Fig. 18). Larvae can then be examined in the field or taken back to the laboratory to be dissected or reared to detect parasitoids (see Chapter 6 for pictures and descriptions of parasitoid life stages). Several studies have demonstrated the effectiveness of this method (Duan et al., 2012b, 2013a,b, 2014).

Advantages and Disadvantages of Methods for Detection of Larval Parasitoids

Similar to the use of sentinel eggs, deployment of sentinel larvae requires substantial infrastructure and manpower. EAB must be reared from the egg to 3rd or early 4th instar larval stage to be suitable for use. Predation of sentinel larvae is not a problem, but bacterial or fungal contamination can be, and once introduced into colonies, pathogens can become pervasive and difficult to eliminate. Additionally, un-infested ash is needed both for rearing EAB larvae and creating LSL units. Finding un-infested ash of the

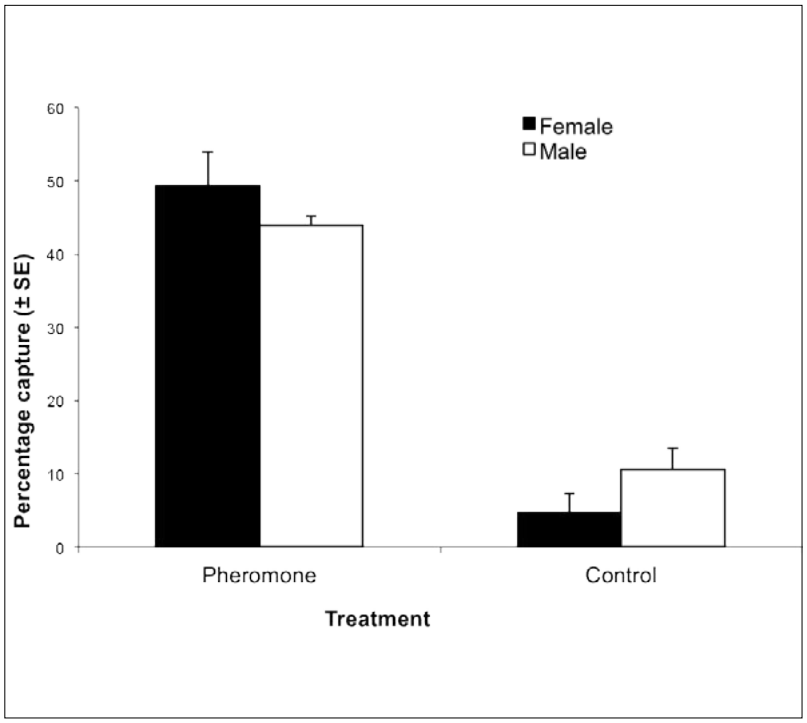


Figure 16. Percentage (± SE) of captured virgin male and female *Spathius agrili* on yellow sticky traps baited with pheromone.

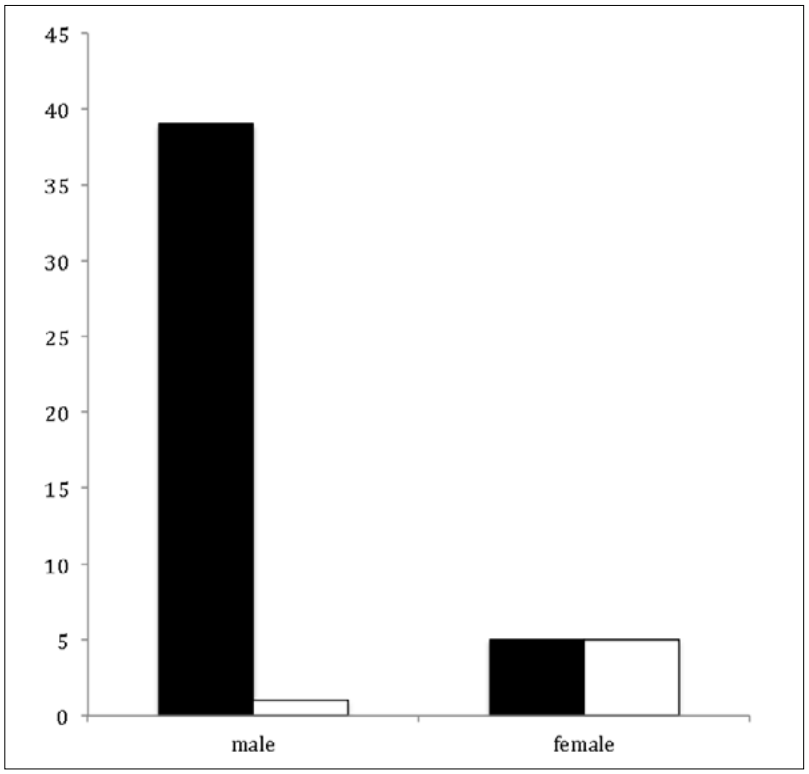


Figure 17. Total number of trapped male and female *Tetrastichus planipennisi* on yellow pan traps baited with pheromone.

Figure 18. Peeling bark from ash trees to search for naturally occurring emerald ash borer larvae. (Photo credit: Leah Bauer)

appropriate size can be difficult, especially in regions where EAB is abundant. Also, native parasitoids that attack EAB, such as *Atanycolus* spp. (Hymenoptera: Braconidae), sometimes attack many of the sentinel larvae, thus preventing assessment of introduced parasitoids. Despite these disadvantages, sentinel larvae allow for standardized, nondestructive detection of larval parasitoids. In addition, sentinel larvae can be deployed at any time during the field season. It is important, however, to deploy them when larval parasitoids are likely to be present (May-September).

Sampling naturally occurring EAB larvae by peeling the bark of infested trees eliminates the need to rear and maintain EAB larvae, which must be done for sentinel larval methods. It also allows for the direct assessment of what is currently occurring in the field and estimation of attack rates by larval parasitoids. Bark peeling is destructive, however, so unlike the use of sentinel larval methods, in plots where there is a need for repeated sampling, collection of wild larvae must be limited to preserve trees for future work. Peeling bark is laborious and requires careful technique to avoid damaging larvae, since damaged larvae are often difficult to diagnose for parasitism, particularly for ectoparasites like *Spathius* spp. and *Atanycolus* spp., which can easily be dislodged and lost. In addition, cases in which larval parasitoids have already emerged are often difficult to diagnose. Finally, woodpecker predation can be high (Lindell et al., 2008; Duan et al., 2012b, 2013a, 2014; Jennings et al., 2013), and it is not possible to determine if larvae taken by woodpeckers were also parasitized or not.

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On the cover: Cover design by Sheryl Romero and Denise Binion, Forest Health Technology Enterprise Team. Background image: Understory green ash seedlings (*Fraxinus pennsylvanica*, Oleaceae) released after large ash trees were killed by emerald ash borer in Okemos, Michigan in 2014, photo by Leah S. Bauer; (bottom row, left to right) Fully mature *Tetrastrichus planipennisi* larvae break free of emerald ash borer larval skin and pupate in the larval gallery under the tree bark. (Photo credit: Clifford Sadof); EAB adult and typical leaf feeding damage. (Photo credit: Deborah Miller, USDA Forest Service, Bugwood.org); Emerging *Tetrastrichus plannipennisi* adults. (Photo credit Leah S. Bauer).

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