Chapter 5: History of Emerald Ash Borer Biological Control

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INTRODUCTION

The search for natural enemies of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in northeastern Asia, its native range, was initiated within a year of its discovery in the United States (Bauer et al., 2005, 2014). Although the official response to EAB's invasion in both the United States and Canada was to regulate and attempt to eradicate EAB, the size and complexity of the outbreak prompted scientists and policy makers to support exploration for natural enemies as a potential tool for management of EAB. In 2008, when populations of EAB were already known to occur in nine states, the U.S. government moved from a policy of eradication to one of management (USDA-APHIS, 2013). By this time, scientists had completed all the steps necessary to secure permits for field release of three EAB natural enemies (host range assessment and safety evaluations) from China, and the use of these parasitoids was incorporated into the EAB management plan. This chapter documents the considerable efforts that went into making this possible in just five years.

EAB NATURAL ENEMIES FROM THE UNITED STATES

Before pursuing classical biological control, it is important to determine if locally present natural enemies are attacking and affecting populations of the pest in the invaded area. After the discovery of EAB in Michigan in 2002, studies were conducted in Michigan and Pennsylvania that found several larval parasitoids but no egg parasitoids (Bauer et al. 2004, 2005; Duan et al. 2009, 2013a). Most of these larval parasitoids are associated with native species of *Agrilus* (Taylor et al., 2012). Parasitism rates measured soon after the discovery of EAB in the United States were low (<1% in Michigan and 5% in Pennsylvania) compared to rates seen in Asia (Liu et al., 2003, 2007; Yang et al., 2005; Duan et al., 2012a) and rates reported in the literature for native *Agrilus* spp. in the United States. This low level of natural enemy attack on EAB was clearly inadequate to suppress EAB populations. Entomopathogenic fungi caused about 2% mortality of EAB life stages under the bark (Bauer et al., 2004). Predaceous beetles and woodpeckers also attacked EAB, but not in sufficient numbers to significantly affect EAB densities (Bauer et al., 2004; Lindell et al., 2008; Duan et al., 2011; Jennings et al., 2013). The lack of natural enemies capable of suppressing EAB populations below a density permitting survival of native ash trees was of great importance (Federal Register, 2007), and this risk supported the decision to introduce parasitoids that coevolved with EAB in Asia for biological control of EAB in North America (Bauer et al., 2008).

EXPLORATION IN ASIA FOR EMERALD ASH BORER NATURAL ENEMIES

Natural Enemy Surveys in China

Initially, exploration for EAB natural enemies focused on China (Liu et al., 2003), investigating 11 locations in six areas where EAB had previously been reported: the provinces of Heilongjiang, Jilin, Liaoning, Hebei, and Shandong, as well as the city-province of Tianjin (Yu, 1992; Xu, 2003). EAB was found in all provinces except Shandong (Liu et al., 2003). By 2002, these surveys in China had identified two larval parasitoids of EAB, *Spathius* sp. (Hymenoptera: Braconidae) and *Tetrastichus* sp. (Hymenoptera: Eulophidae) (Liu et al., 2003), and later in 2004, one egg parasitoid, *Oobius* sp. (Hymeoptera: Encyrtidae) was found (Zhang et al., 2005). These species were later described as *Spathius agrili* Yang (Yang et al., 2005), *Tetrastichus planipennisi* Yang (Yang et al., 2006), and *Oobius agrili* Zhang and Huang (Zhang et al., 2005).

In depth studies of the population dynamics of EAB and its natural enemies were carried out in Jilin and Liaoning Provinces in northeastern China in 2004 and 2005. The two most abundant natural enemies of EAB, collected from EAB-infested *Fraxinus pennsylvanica* Marshall (native to eastern North America) in Jilin Province, were the larval parasitoid *T. planipennisi* and the egg parasitoid *O. agrili* (Liu et al., 2007). During the course of this study, these two parasitoid species reduced EAB densities by an estimated 74% in the infested trees. *Tetrastichus planipennisi* is also known from other provinces in northeast China, including Hebei (LSB unpublished data) and Heilongjiang (Yang et al., 2006).

Tetrastichus planipennisi is a gregarious endoparasitoid that attacks EAB larvae by drilling through the tree bark with its ovipositor. Brood sizes (number of eggs laid per host larva) range from 4 to 172 (Yang et al., 2006; Ulyshen et al., 2010). The EAB larva continues feeding as parasitoid larvae develop inside its body; eventually the parasitoids consume most of the EAB larva and emerge into the gallery, where they pupate and develop into adults. Adult wasps chew through the bark and emerge. There are up to four such generations per year. Average parasitism of larvae by *T. planipennisi* in China was 22.4%, but reached up to 65% (Liu et al., 2003, 2007; Yang et al., 2006).

Oobius agrili is a small (1 mm long), parthenogenic wasp that lays its eggs singly inside EAB eggs. In general, adults from the first generation emerge to attack more EAB eggs, while wasps of the second generation enter diapause and overwinter inside the host egg. However, some individuals of the first generation of *O. agrili* do not emerge until the following spring. Parasitism of EAB eggs was as high as 61.5% by the end of the field season at some locations in northeastern China (Liu et al., 2007).

Although scarce in northeast China, *S. agrili*, the third EAB parasitoid from China, was most abundant in Tianjin City in planted stands of *Fraxinus velutina* Torr. (native to the southwestern North America) (Xu, 2003; Liu et al., 2003; Yang et al., 2005). Parasitism of EAB larvae by *S. agrili* was as high as 90% in some stands in Tianjin by the end of the season (Yang et al., 2010). *Spathius agrili* females use their ovipositor to drill through the bark into the EAB larva, inject venom to paralyze it, and lay an average of five eggs per host larva (Gould et al., 2011). The parasitoid larvae hatch and feed externally on the EAB larva. Mature parasitoids pupate inside cocoons in the gallery, and adult wasps chew through the bark to emerge. Yang et al. (2010) estimate that *S. agrili* completes 3-4 generations per year in Tianjin.

A fourth species of parasitic wasp, in the genus *Sclerodermus* (Hymenoptera: Bethylidae), was discovered parasitizing mature EAB larvae and pupae in Tianjin (Wu et al., 2008) and later described as *Sclerodermus pupariae* Yang et Yao (Yang et al., 2012). After locating a host, female wasps chew through the bark and use their strong front legs to excavate a tunnel through the tightly packed EAB frass in the host gallery to locate host larvae or pupae. They then sting and paralyze their host, feed on the hemolymph, and lay an average of 40 eggs per host. After hatching, *S. pupariae* larvae feed externally on the host and, with some maternal care during the larval stage, pupate inside cocoons. Approximately 13% of the EAB sampled in Tianjin were parasitized by *S. pupariae* (Yang et al., 2012).

Natural Enemy Surveys in the Russian Far East

Exploration for EAB parasitoids in Russia was concentrated near Vladivostok and Khabarovsk, where EAB is native (Williams et al., 2010; Duan et al., 2012a). As in China, ash trees native to Asia (*F. mandshurica* Rupr. and *F. chinesis* Roxb. subsp. *rhynchophylla*) and to North America (*F.*

pennsylvanica) were sampled in both natural forests and urban areas. Little parasitism was noted in Khabarovsk, but three larval parasitoid species were recovered in the Vladivostok region (Duan et al., 2012a), as well as a strain of egg parasitoid in the genus *Oobius* that completes one generation per year and, based on DNA evidence, appears to be a different species than *O. agrili* from China (JJD, unpublished data). The larval parasitoids, mainly attacking EAB in *F. pennsylvanica*, included *T. planipennisi, Atanycolus nigriventris* Vojnovskaja-Krieger, and a previously unknown species of *Spathius*. The latter species was recently described as *Spathius galinae* Belokobylskij, and although its general biology is similar to that of *S. agrili,* it has a longer ovipositor and may be better adapted to the cold climate of the north central United States (Belokobylskij et al., 2012). Depending on the site and year, parasitism rates were approximately 24% for *T. planipennisi*, 23% for *A. nigriventris*, 76% for *S. galinae*, and 28% for *O. agrili* (Duan et al. 2012a; JJD unpublished data).

Natural Enemy Surveys in South Korea

Emerald ash borer is quite rare in South Korea and is probably kept in check by a combination of host resistance and natural enemies (Williams et al., 2010). After several years of exploration, two EAB populations were discovered attacking *F. chinesis* subsp. *rhynchophylla* and *F. mandshurica*. One EAB population was found in stressed landscape trees near the city of Daejeon, and the other was on trees damaged during construction further north at a site near Seoul. At these sites, three natural enemy species were discovered: a larval ectoparasitoid later identified as *S. galinae,* a larval endoparasitoid tentatively identified as *Tetrastichus telon* Graham, and the clerid beetle *Teneroides maculicollis* Lewis. The clerid attacked EAB in the overwintering pupal chamber, where it pupated after consuming the host. The three species were brought to a United States quarantine facility; however, colonies could not be established.

Surveys for Natural Enemies in Mongolia/Japan

Although *A. planipennis* has been reported from Japan (Schaefer, 2004) it is quite rare (Haack et al., 2002). A Japanese buprestid specialist returned to a locality from which *A. planipennis* had previously been collected and found a single adult beetle on a leaf. In Fukui Prefecture, Honshu, Japan, EAB is listed as endangered because only two collection locations have been recorded. Natural enemies of EAB were not recovered in Japan (Schaefer, 2005).

Foreign exploration in Mongolia was even more fruitless (Schaefer, 2005). Not only were no EAB populations found, but collectors could not even find ash trees. No species in the genus *Fraxinus* occur in the published list of Mongolian vascular plants, and *A. planipennis* has not been recorded from Mongolia. Schaefer (2005) hypothesized that someone may have erred and associated collection of EAB from Mongolia when the discovery may instead have been in Inner Mongolia, China, where EAB is thought to occur.

SELECTION OF POTENTIAL BIOLOGICAL CONTROL AGENTS

Just because a natural enemy is found attacking the target pest in its native range does not necessarily make it suitable for use as a biological control agent (González and Gilstrap, 1992). *Sclerodermus pupariae* has several features lowering its potential as a biological control agent: (1) many females lack wings and would not disperse well, (2) the percentage parasitism observed in China was low, (3) it had a broad host range, and (4) members of the genus are known to sting humans (Gordh and Maczar, 1990; Tang et al., 2012; Yang et al., 2012; Wei et al., 2013). Therefore, this species was not considered for importation as a potential biological control agent for EAB. In contrast, *A. nigriventris* has better potential for use against EAB; however, scientists have yet to succeed in getting them to mate in the laboratory (JJD personal communication). Host specificity testing would also need to be conducted carefully because *Atanycolus* species native to the United States tend to have a broad host range and are known to attack EAB, often in large numbers (Cappaert and McCullough, 2009).

Oobius agrili, *T. planipennisi*, *S. agrili*, and *S. galinae* were all considered promising candidates for biological control. All four species possess characteristics considered by Kimberling (2004) as enhancing the likelihood of successful biocontrol: female-biased sex ratio or parthenogenesis, a short generation time, and high rates of parasitism and fecundity. These four species were imported into quarantine in the United States for host range testing.

Quarantine Screening

Rearing EAB and its parasitoids. Before scientists could study the biology and host preferences of EAB parasitoids, it was necessary to develop methods to rear both EAB and the parasitoids (see Ch. 8). There were several challenges that needed to be met: (1) parasitoids can potentially be reared all year but EAB is univoltine and has an obligatory diapause as mature larvae, (2) adult EAB eat ash leaves, but leaves are only available in the field in the summer, (3) EAB eggs are needed for rearing the egg parasitoid *O. agrili*, and (4) the larval parasitoids only attack EAB when it is beneath ash bark.

The dilemma of EAB availability was solved by felling ash trees containing large numbers of EAB and storing the logs in cold rooms until the insects were needed. Felling of ash trees could be done (1) during the late summer when logs contained mature larvae appropriate for rearing larval parasitoids or (2) during the winter when overwintering mature fourth-instar larvae were present in their pupation cells (as J-larvae), which quickly developed into EAB adults when warmed.

The need to obtain foliage to feed adults throughout the year was solved by rearing tropical or Shamel ash, *Fraxinus uhdei* (Wenz.) Lingelsh., in greenhouses.

Initially, small ash logs wrapped in curling ribbon were presented to adult beetles for oviposition, but a method of coaxing females to lay eggs on coffee filters was later developed. Eggs on filter papers could then be presented to *O. agrili* for parasitization.

EAB larvae were extracted from the logs by peeling the bark, and these larvae were reinserted in grooves under bark flaps of small ash logs for presentation to *S. agrili* and *T. planipennisi* (Gould et al., 2011). This method was laborious and was later improved upon for mass production (see Ch. 8).

Host specificity testing. Biological control of insect pests using entomophagous natural enemies has generally been considered "natural" and "safe." However recent studies have documented negative impacts on non-target species in some cases (Boettner et al., 2000; Obrycki et al., 2000; Henneman and Marmot, 2001), highlighting the need for pre-release host specificity testing. The specificity of EAB parasitoids imported from China or Russia was estimated in quarantine as part of the process of assembling data needed to apply for release permits. Specificity of agents is summarized below.

(1) Spathius agrili. No-choice host specificity tests with *S. agrili* were conducted in China and the United States to determine possible direct effects on non-target species (Yang et al., 2008). *Spathius agrili* finds hosts to parasitize by hearing sounds or feeling vibrations produced by feeding larvae inside wood. All test larvae, therefore, were presented while feeding inside their natural host trees. In initial no-choice host specificity tests, *S. agrili* did not parasitize wood-boring Lepidoptera, a longhorned beetle (Cerambycidae), or the one *Agrilus* species tested. Of these three species (whose larvae all attack ash), only EAB was parasitized. Further testing was, therefore, confined to members of the genus *Agrilus*, which were hypothesized to potentially be at risk because they were closely related to EAB.

In the United States, we tested the two-lined chestnut borer, *Agrilus bilineatus* Weber, in oak (*Quercus*), and the bronze birch borer, *Agrilus anxius* Gory, in birch (*Betula*), while in China various other local *Agrilus* species were tested.

In no-choice tests, *S. agrili* attacked some species of *Agrilus* other than EAB, but at rates that were significantly lower than for emerald ash borer. In China, *S. agrili* attacked *Agrilus zanthoxylumi* Hou, *Agrilus mali* Matsumura, and *Agrilus inamoenus* Kerremans. No attack occurred on other *Agrilus* species tested, *Sphenoptera* sp. (Coleoptera: Buprestidae), or *Eucryptorrhynchus chinensis* (Olivier) (Coleoptera: Curculionidae) (Yang et al., 2008).

No-choice tests determine the physiological host range of a parasitoid by giving them no other option but to oviposit on a non-target host. Parasitoid adult orientation to host plants is not part of the test and so this filter is disregarded. To determine the ecological host range of *S. agrili*, olfactometer tests with adults were conducted in China to determine if *S. agrili* were attracted to the plant species harboring the larvae tested in no-choice tests. Naïve, mated *S. agrili* females were placed in vertical y-tube olfactometers and given a choice of leaves and twigs of various host plants or clean air. *Spathius agrili* was only attracted to two ash species (*F. pennsylvanica* and *F. velutina*) and one species of willow (*Salix babylonica* L.) (Yang et al., 2008). Even though some attack occurred on larvae found in *Citrus reticulata* Blanco, *Malus micromalus* Makino, and *Zanthoxylum bungeanum* Maxim in no-choice tests, *S. agrili* females were not attracted to these tree species. In nature, if parasitoids are not attracted to an insect's host tree, they would be unlikely to encounter and parasitize larvae of that non-target species. *Spathius agrili* was attracted to willow leaves, and at least three *Agrilus* species attack willow in the United States: *Agrilus pratensis pratensis* Ratzburg (adults 4 - 6 mm long), *Agrilus politus* Say (adults 5.0 - 8.5 mm long), and *Agrilus quadriguttatus* Gory. These insects are quite small compared with adult EAB, which are 8.5-13.5 mm long. *Spathius agrili* attacks only large EAB larvae, and even mature larvae of *A. pratensis* and *A. politus* are likely too small to be at risk of attack. Thus even if *S. agrili* is attracted to willow in the United States, it is unlikely to encounter any non-target species large enough to be suitable hosts.

Another piece of evidence concerning host specificity was gathered in China by collecting larvae of six *Agrilus* species in the field and then rearing them to determine their parasitoid fauna. A total of 2,074 *Agrilus* larvae of six non-target species were collected and neither *S. agrili* nor *T. planipennisi* were recovered (Yang et al., 2008). Given the combination of evidence from no-choice tests (lower parasitism rates or no attack on non-target *Agrilus* species), olfactometer tests (only attracted to ash and willow), and the lack of *S. agrili* reared from other *Agrilus* species in China, it was predicted that release of *S. agrili* would not have adverse direct effects on non-target species in the United States.

(2) Tetrastichus planipennisi. To evaluate the direct effects of *T. planipennisi* on potential non-target North American insect species, no-choice assays were performed in the laboratory with larvae of EAB and eight species of buprestids (five species of *Agrilus* and three of *Chrysobothris*), five cerambycids, two lepidopterans, and one hymenopteran (Liu and Bauer, 2007; Federal Register, 2007). These insects were selected based on (1) the degree of taxonomic closeness to EAB; (2) overlap in habitat and/or niche with EAB; (3) risk to beneficial, threatened, or endangered insects; and (4) feasibility of acquiring or rearing enough larvae to perform replicated assays. *Tetrastichus planipennisi* did not attack any of the seventeen non-target species presented in no-choice tests, it was considered quite host specific, and further testing was not done.

(3) Oobius agrili. To evaluate the direct effects of *O. agrili* on non-target insect species, no-choice assays were performed in the laboratory using eggs of six *Agrilus* species, two cerambycids, and four lepidopterans (Bauer and Liu, 2007; Federal Register, 2007). In no-choice assays, *O. agrili* did not oviposit in eggs of the cerambycids or lepidoptera. *Oobius agrili* may oviposit and develop in *Agrilus* eggs from different species if they have eggs similar in size to those of EAB. Such non-target species include *A. anxius* (bronze birch borer), *A. bilineatus* (two-lined chestnut borer), and *Agrilus ruficollis* (F.) (red-necked cane borer), which are pests of birch, oak, and raspberry, respectively. Paired no-choice and choice assays were then performed for two of the *Agrilus* species that were accepted by *O. agrili* during the no-choice assays. In the choice assays, *O. agrili* preferred eggs of *A. planipennis* (EAB) on ash logs over those of *A. anxius* or *A. ruficollis,* on birch and raspberry, respectively.

(4) Spathius galinae. To evaluate the effects of *S. galinae* on non-target insect species, no-choice and choice host specificity tests were conducted (JRG and JJD unpublished). Fifteen North American species of wood-boring insects were exposed to *S. galinae* to assess the parasitoid's physiological host range. Emphasis was placed on species closely related to the target pest or those feeding on ash. Thirteen of these fifteen species were wood-boring beetles, one was a clearwing moth (Lepidoptera: Sesiidae) and one a sawfly (Hymenoptera: Cephidae). Of the beetles, five were in the genus *Agrilus,* and were thus closely related to the EAB, and another was in the same family (Buprestidae). Three of the insects tested – the longhorned beetle *Neoclytus acuminatus* (F.), the clear-winged moth (*Podosesia* sp.), and the eastern ash bark beetle, *Hylesinus fraxini* Panz. (Coleop.: Scolytinae) – attack ash as their main host and would be susceptible to parasitism if *S. galinae* accepts any boring insects infesting ash.

Spathius galinae attacked only one species other than the EAB, the gold spotted oak borer (*Agrilus auroguttatus* Schaeffer) in red oak (*Quercus rubra* L.). This species is an invasive borer killing native oaks in California. The rate of parasitism was, however, lower (only 41%) on the non-target host compared to EAB (71%) under test conditions that strongly favored parasitism. *Spathius galinae* did not attack the other three test species that infested red oak, nor did it attack the other *Agrilus* or the three non-*Agrilus* species infesting ash. This level of host specificity is quite high; indeed, higher than that of *S. agrili,* which was approved for release against EAB.

Applying for Release Permits

Applying for permits to release exotic parasitoids against invasive pests in the United States is a complicated process involving review by the North American Plant Protection Organization (NAPPO), whose members include the United States, Canada, and Mexico, by the U.S Fish and Wildlife Service, North American Indian Tribes, the USDA-APHIS permitting unit, and State Departments of Agriculture (Bauer et al., 2014). The application must also be posted in the Federal Register for public comment before APHIS approves or disapproves the application. In January, 2007, USDA scientists applied for environmental release permits for *S. agrili*, *T. planipennisi*, and *O. agrili* in Michigan. The permits were granted at the end of July 2007, and the parasitoid species were released at several sites in Michigan. The application to release *S. galinae* was submitted in March, 2013 and in November of that year was approved by the NAPPO committee. The final outcome of the permit application to release *S. galinae* in the United States was pending in January 2015.

FIELD ESTABLISHMENT AND EVALUATION OF NATURAL ENEMIES

Rearing and Release

After release permits were issued in 2007, relatively small numbers of adult *S. agrili*, *T. planipennisi*, and *O. agrili* were released at field sites in Michigan. Releases were expanded to new sites in Ohio and Indiana in 2008, and to Illinois and Maryland in 2009, but only a few hundred adults of each species were released because of the limited rearing capacity of the USDA research laboratories. Despite these limitations, however, establishment of the three species was confirmed within two years at many of these field sites (Bauer et al., 2008, 2011). These early successes resulted in the decision by USDA to initiate an EAB Biological Control Program in 2009 and construct the APHIS EAB Biocontrol Facility in Brighton, Michigan (USDA, 2013). Researchers wrote guidelines to assist land managers with basic information on EAB, the biological control agents, site selection, and methods for release and recovery of the parasitoids (Gould et al., 2013). An online database was also developed where the Guidelines are posted and parasitoids release and recovery data are entered and mapped (mapbiocontrol.org). The EAB biological control agents are now mass-reared for distribution and release throughout the still expanding EAB infestation in North America, and production and release methods continue to be improved (see Ch. 8).

In 2009, the APHIS EAB Biocontrol Facility concentrated on rearing *S. agrili*, and 10,000 adult females of that species were reared and released (Fig. 1) (J. Lelito, personal communication). In 2010, a concerted effort was made to increase production of *T. planipennisi* (Fig. 2), and production of the egg parasitoid, *O. agrili,* was greatly increased in 2011 (Fig. 3). Production of these parasitoids increased

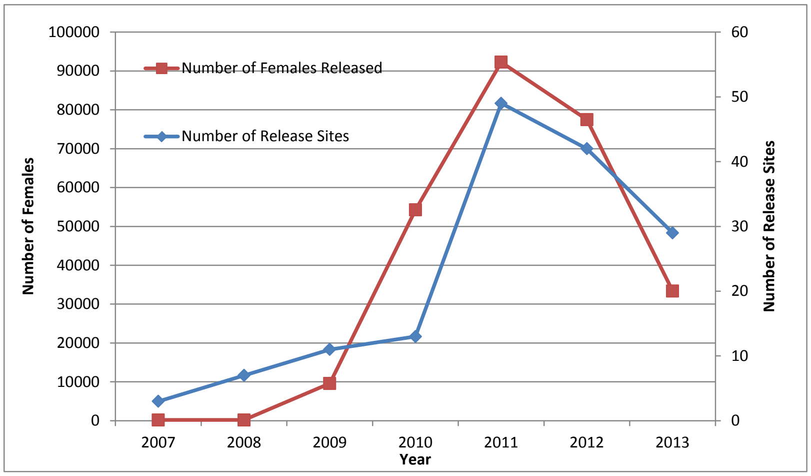

Figure 1. Number of female *Spathius agrili* released against emerald ash borer 2007-2013 and the number of release sites in the United States.

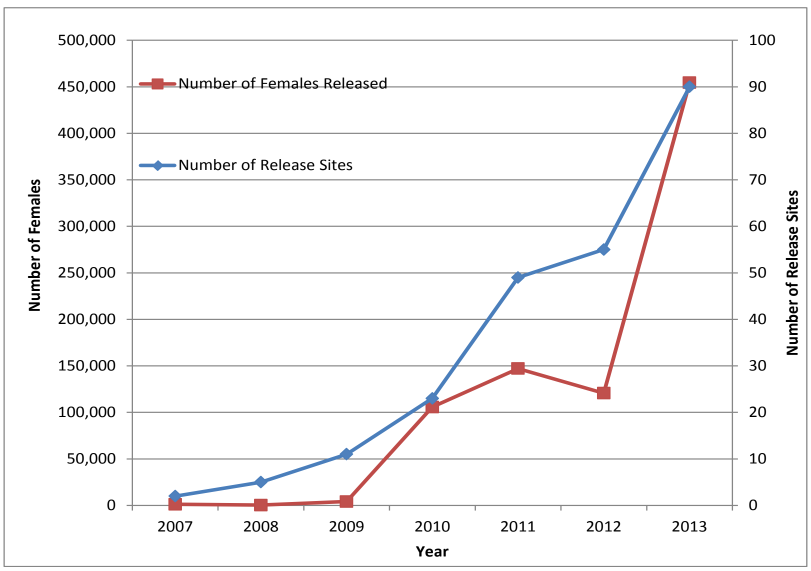

Figure 2. Number of female *Tetrastichus planipennisi* released against emerald ash borer 2007-2013 and the number of release sites in the United States.

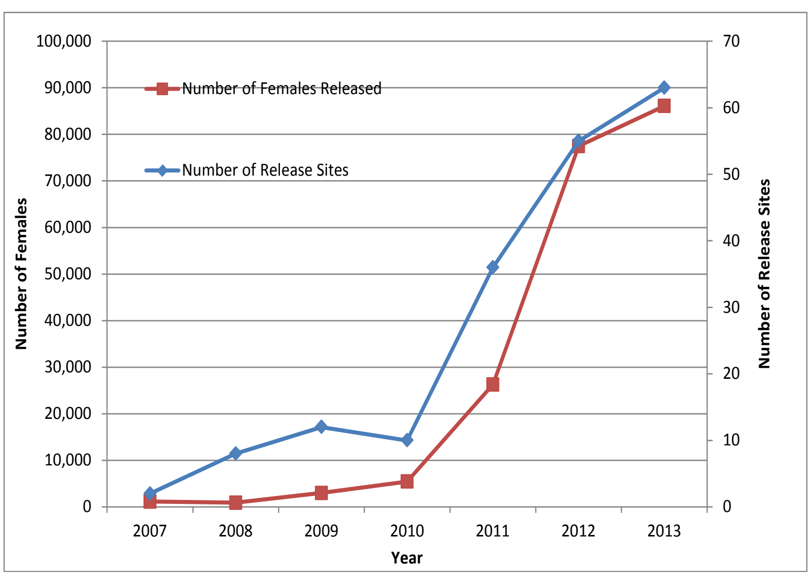

Figure 3. Number of female *Oobius agrili* released against emerald ash borer 2007- 2013 and the number of release sites in the United States.

in subsequent years, with the exception of *S. agrili*, which was not released in northern states after 2012 (see the Establishment section for explanation). The increased production was necessary because each year at least two more states initiated releases of EAB parasitoids (Table 1), so that by 2013 seventeen states were conducting releases. In 2014 Colorado, Georgia, New Hampshire, and New Jersey discovered populations of EAB and releases in those states were set to begin that year.

To improve parasitoid production and release efficiency, the APHIS EAB Biocontrol Facility began modifying the shipment and release methods for the three EAB biological control agents. Before 2012, parasitoids were released as adults. This was done by rearing them to the adult stage in the laboratory, consolidating them into plastic cups provisioned with honey, and shipping them inside coolers to cooperators who released them onto the trunks of EAB-infested ash trees. In 2012, APHIS began shipping parasitoids as mature larvae or pupae for self-emergence in the field from cups containing parasitized EAB eggs for *O. agrili* or ash bolts containing EAB larvae parasitized with either *T. planipennisi* or *S. agrili*. This change began for *O. agrili* in 2012 and for the two larval parasitoid species in 2013. Further research is needed to evaluate the success of the newer release methods.

Table 1. States initiating releases of EAB parasitoids by year.

Year	States Inititiating Releases
2007	Michigan
2008	Ohio, Indiana
2009	Illinois, Maryland
2010	West Virginia, Kentucky, New York, Wisconsin
2011	Pennsylvania, Minnesota, Virginia
2012	Missouri. Tennessee
2013	Massachusetts, North Carolina, Connecticut

Establishment

Sampling methods. Sampling EAB to determine whether or not larvae or eggs are parasitized poses quite a challenge. EAB eggs are small and laid between layers of bark and in bark crevices. EAB larvae feed beneath the bark of ash trees, and to recover them the bark must be peeled off to expose the larvae. If the goal is to determine the percentage of EAB that are parasitized, one must search ash trees for EAB eggs and larvae.

However, if the goal is only to confirm establishment, several additional methods have been developed (Bauer et al., 2012; Duan et al., 2011, 2012b). For detection of *O. agrili*, laboratory-reared EAB eggs can be placed in the field under bark flaps, on small ash logs, or in cups on paper. For the larval parasitoids, EAB larvae can be inserted in small ash logs and placed in the field. Creating these “sentinel” eggs or larvae, however, requires either rearing adult EAB or collecting larvae for insertion in the sentinel logs. EAB parasitoids have been recovered at several sites using sentinel logs, and egg sentinel logs were used to document the phenology of *O. agrili* activity in the field in Michigan (Abell et al., 2011).

Both larval and egg parasitoids can also be recovered by collecting logs or bark samples and placing them in cardboard rearing tubes fitted with collection jars. The emerging parasitoid adults are attracted to the light in the jar and essentially collect themselves.

Finally, adult parasitoids can be recovered in the field using yellow pan traps filled with a solution of propylene glycol (Bauer et al., 2013). These traps are inexpensive to produce and easy to deploy, but distinguishing the biological control agents from similar native species requires individuals trained in insect taxonomy and identification.

Pheromones have been identified for both *S. agrili* and *T. planipennisi* (Bauer et al., 2011; Cossé et al., 2012), and the use of pheromone lures is being investigated as a method to increase the efficacy of yellow pan traps.

Reproduction, overwintering, and establishment. For the introduced parasitoids to successfully control EAB, they must find conditions suitable for reproduction in the field, survive cold winter temperatures, and persist from year to year. The three introduced parasitoids released as EAB biological control agents have several generations per year, and their presence in the field must coincide with the availability of the stages suitable for parasitism (eggs for *O. agrili* and larvae for *S. agrili* and *T. planipennisi*). Throughout their adult lives, the parasitoid must also find sources of nourishment such as nectar or honeydew.

Spathius agrili was found parasitizing 18% of the EAB larvae sampled the spring following the release of 175 females in 2007 in southern MI (JRG unpublished). However, samples collected from 40 ash trees the following year revealed not a single parasitoid. At another site, parasitism one year after release was 45%. Two broods were discovered each of the following two years, but parasitism by *S. agrili* remained consistently low. At six more intensively sampled study sites in Michigan, *S. agrili* was recovered in yellow pan traps, but only two EAB larvae parasitized by this species were recovered after 2-5 years of sampling (Duan et al., 2013b). *Spathius agrili* also does not seem to have persisted in Maryland or Ohio. One possible explanation for this apparent lack of persistence is that the population of *S. agrili* reared for release in the United States originated from Tianjin, China. The latitude of this city is near the 39th parallel, and the climate there is a better match for the central (north-south) rather than the northern United States. *Spathius agrili* can successfully overwinter in the midwestern United States, so cold is probably not the limiting factor. Perhaps there is a problem with synchrony between the emergence of adult *S. agrili* and availability of the mature EAB larvae that they need to attack. Based on this observation, the EAB Biocontrol Program decided in 2013 to cease releasing *S. agrili* above the 40th parallel in North America. If *S. galinae* is approved for release, we anticipate that it will be better synchronized with its EAB host in the more northern states. *Spathius agrili* has also been recovered in Illinois, Indiana, Pennsylvania, New York and Tennessee, and we are especially interested in whether *S. agrili* will persist in Tennessee, where the climate is more similar to the parasitoid's native range in China.

Oobius agrili has been recovered in Michigan, Ohio, Indiana, Pennsylvania, Maryland, and New York. At two sites in Michigan, parasitism by *O. agrili* increased from 5% one year following release to 20% two years later (Duan et al., 2011, 2012b; Abell et al., 2011). This species was also recovered at non-release locations at least 800 m from the release site two years after release. Parasitized eggs were found on 73% of trees in the release plots and 25% of the trees in the control plots (Abell et al., 2014), providing evidence that *O. agrili* populations are slowly building and dispersing in Michigan.

Establishment and spread of *T. planipennisi* is even more impressive. At six intensively studied sites in Michigan, 92% of the trees at the release sites contained at least one brood of *T. planipennisi* four years after release, and parasitism levels increased steadily to an average of over 20% (Duan et al., 2013b). Parasitism by *T. planipennisi* at the six control sites (at least 1 km away) also increased yearly to an average level of 13% after four years. The rearing facility in Brighton, Michigan, often finds *T. planipennisi* in trees harvested far from known release locations. It is not known whether *T. planipennisi* is dispersing so well on its own or by human movement of infested firewood, or both. *Tetrastichus planipennisi* has also been recovered from Illinois, Indiana, Ohio, New York, Maryland, Wisconsin, and Minnesota.

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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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