## Progress on Biological Control of the Emerald Ash Borer in North America



In 2002, the emerald ash borer (EAB), *Agrilus planipennis,* a buprestid beetle native to Northeast Asia, was discovered as the cause of extensive ash tree (*Fraxinus* spp.) mortality in southern Michigan and Ontario. It is believed that EAB was introduced into Michigan from solid-wood packing materials during the 1990s and became established in the abundant ash resources throughout urban areas, parks, forests, and riparian ecosystems[1]. In an effort to protect ash resources in North America, regulatory agencies imposed quarantines and developed programmes to eradicate and contain EAB. Although these efforts may have slowed the rate of spread, eradication was abandoned in the 'core infestation' due to the size of the infestation, the lack of effective methods to detect and control EAB, and the challenges associated with quarantine compliance and enforcement. Although researchers estimate EAB can spread 10 to 20 miles (16–24 km) per year, humans are responsible for its long-range dispersal through the illegal transport of infested ash nursery stock, firewood, and logs. Regulatory agencies have determined that EAB is established throughout Lower Michigan and areas of the neighbouring states of Ohio and Indiana; separate infestations are

also known in Michigan's Upper Peninsula, Illinois, Maryland, and Pennsylvania. This invasive buprestid is expected to continue spreading throughout North America due to the widespread use of ash as a landscape tree and the prevalence of ash trees throughout forested and riparian ecosystems. Land managers and the public are now seeking sustainable management methods to reduce EAB population densities and to slow its rate of spread.

When EAB was discovered in North America, only scant information on its biology was available in the literature from Asia where it is considered only a minor and periodic pest of indigenous ash species. To develop biological and microbial controls for EAB in North America, we began studying this beetle in Michigan and China in 2002. EAB completes its life cycle in one or two years depending on the age of the infestation, tree health, and other biotic and abiotic factors. Emergence of EAB adults typically begins in late May and peaks during June. Adults chew D-shaped exit holes in the tree bark to emerge and begin maturation feeding on ash foliage followed by mating; after about three weeks, females start to oviposit in bark crevices and between bark layers. Although egg-laying peaks toward the end of June, eggs are laid throughout the summer and into the fall due to a prolonged adult emergence period and long adult longevity. After egg hatch, EAB neonates tunnel through the tree bark until reaching the phloem where they continue feeding through four larval stages. When mature, EAB larvae chew pupation cells in the outer sapwood or bark; pupation occurs during the spring or summer. Early in an infestation, EAB attacks the upper crown of large ash trees, and as populations increase, the trees become weaker. Tree mortality is caused by larval girdling of the main trunk, when EAB populations reach lethal density thresholds for ash. This occurs over a period of several years depending on initial tree health, species, site, rainfall, and other factors. Current management strategies are being developed with the goals of containing isolated EAB infestations, slowing its spread, and suppressing population densities below a tolerance threshold for survival of North America's ash trees.

### *Lack of Natural Enemies in North America*

We conducted natural enemy studies in Michigan from 2002 to 2004, and found 0.7% parasitism for EAB larvae; no egg parasitoids were found. The larval parasitoids reared from EAB larvae in Michigan included hymenopteran parasitoids that attack native *Agrilus* spp: two native braconids (*Atanycolus* sp. and *Spathius floridanus*), one native chalcid (*Phasgonophora sulcata*), and one exotic eupelmid (*Balcha indica*). This rate of parasitism detected for EAB in the USA is low compared to EAB parasitism rates found in China and those reported in the literature for native *Agrilus* spp. Other biological factors causing EAB mortality include entomopathogenic fungi, three predatory beetle species (*Enoclerus* sp., *Tenebroides* sp. and *Catogenus rufus),* and woodpeckers, but rates of attack were relatively low and constant at different EAB population densities. Some mortality resulted from larval starvation, dehydration, and cannibalism in over-infested trees. The lack of natural enemies capable of suppressing EAB populations below a density threshold tolerable for survival of native ash trees is especially troubling, and supports the need to introduce parasitoids that coevolved with EAB in Asia for biological control of this buprestid in North America.

### *Natural Enemies of EAB in China*

Foreign exploration for EAB in its native range in China resulted in the discovery of three hymenopteran parasitoids considered suitable for use as biocontrol agents in North America: a gregarious larval braconid ectoparasitoid *Spathius agrili*[2,3,4], a gregarious larval eulophid endoparasitoid *Tetrastichus planipennisi*[3,5,6], and a solitary, parthenogenic encyrtid egg parasitoid *Oobius agrili*[6,7]. We have found *T. planipennisi* overlaps in distribution with *S. agrili*[3] and *O. agrili*[6], although additional survey work is needed to identify their distributions and interactions in China.

***Oobius agrili*** was discovered in 2004 in Jilin Province, China[7] although it may be more widely distributed. In China, *O. agrili* is a solitary egg parasitoid with at least two generations per year. It spends the winter and spring as a mature larva in EAB eggs, and adult emergence is synchronized with the EAB oviposition period during July and August in the field. Parasitism rates of *O. agrili* in EAB eggs peak at 61.5% in August. Its distribution overlaps with that of *T. planipennisi* at one of our study sites in China, and the combined mortality of EAB by these two parasitoids is ca. 75%[6]. *Oobius agrili* is parthenogenic, thus females produce females without mating; its sex ratio from field-collected EAB eggs in China is 15:1 (female:male). Much of the knowledge of this parasitoid's biology is based on laboratory studies in our quarantine laboratory. We have found that *O. agrili* females prefer to oviposit in 0- to 6-day-old EAB eggs, live ca. three weeks, and complete a generation every three weeks. The average lifetime fecundity of each female is ca. 24 progeny, with a range of five to 62.

In Michigan, we developed methods to rear *O. agrili* in the laboratory from eggs produced by EAB adults, which are reared from EAB-infested ash logs cut in winter and held in cold-storage. After emergence from infested logs held at room temperature in the laboratory, EAB adults are fed greenhouse-grown *Fraxinus uhdei* foliage and allowed to oviposit on small ash sticks; sticks with EAB eggs are removed and exposed to *O. agrili* for parasitism in a plastic cup with a streak of honey and moist cotton ball. In the laboratory, we performed no-choice assays with eggs of six *Agrilus* spp., two cerambycid beetles, and four lepidopterans. Overlap in physiological host range was found for three native *Agrilus* spp. with eggs of similar size to EAB. For these three species, paired choice assays revealed *O. agrili* strongly preferred to oviposit in EAB eggs laid on ash than in eggs of other *Agrilus* spp. on their respective host plants. This confirms that *O. agrili* is attracted to ash for host location.

***Tetrastichus planipennisi*** was discovered in 2003 in Jilin and Liaoning Provinces of China[3] and later

in Heilongjiang Province[5]. The results of our laboratory and field studies show that *T. planipennisi* oviposits through the tree bark and oviposits into the haemocoel of actively-feeding third- and fourth-instar EAB larvae. It completes at least four generations per year and overwinters as a mature larva inside the host gallery. In the spring, the parasitoid larva pupates, ecloses as an adult, chews a round 1-mm diameter hole in the bark, and the wasp brood exits the tree. An average of 35 parasitoids (range five to 122) emerges from a single EAB larva. During 2005, the results of EAB field research in Jilin Province, China revealed *T. planipennisi* parasitism rates increased from 8% in June to 40% in August[6].

In Michigan, we developed methods to rear *T. planipennisi* in the laboratory by exposing groups of parasitoids to fourth-instar EAB larvae inserted into small ash branches. Adult parasitoids are maintained during the exposure period with a streak of honey and a moistened cotton ball. *T. planipennisi* completes one generation every three weeks and females live about 24 days, twice as long as males. EAB larvae are acquired for parasitoid production by removal from EAB-infested ash logs or after rearing on an ash-based artificial diet. Using our laboratory rearing methods, we evaluated the host specificity of *T. planipennisi* using no-choice assays. In these assays, groups of female and male *T. planipennisi* were exposed to actively-feeding larvae of eight buprestid species, five cerambycid species, and a wood-boring sawfly, all implanted in small branches of their respective host plants. We also assayed larvae of a tenebrionid beetle and two lepidopteran species by implantation in small ash branches, and sphingid larvae by exposure on host leaves. *Tetrastichus planipennisi* rejected all species except actively-feeding EAB larvae implanted in ash branches.

***Spathius agrili*** was first reported in Tianjin, China[2] where it is a prevalent parasitoid of EAB in stands of *Fraxinus velutina*, an ash species native to North America and planted extensively in this part of China. Although *S. agrili* was also found further north in Jilin Province[3], most of the research on this parasitoid was done in Tianjin, which is the southern known range of EAB. In Tianjin, the emergence of *S. agrili* adults was well synchronized with the availability of third- and fourth-instar EAB larvae, its preferred host stages. It completes three generations per year with parasitism increasing from 12% in August to 42% in October[4]. Females oviposit through the tree bark, paralyzing the larva and laying a clutch of eggs on the integument. At maturity, larvae of *S. agrili* spin a cocoon and pupate within the host gallery. Each female lays an average of nine eggs (range two to 18) per clutch, with an average of 23 eggs during her lifetime; average female longevity is 29 days. Laboratory rearing of *S. agrili* is generally similar to that of *T. planipennisi*.

To evaluate the host specificity of *S. agrili*, no-choice assays were conducted in China and the USA. In addition, from 2003 to 2005, potential host larvae from other trees were collected in Tianjin, China where *S. agrili* parasitism of EAB was high. These larvae were then reared to the adult stage and all parasitoids were collected and identified. Because this early testing showed no parasitism of species outside the genus *Agrilus*, even species attacking ash, further testing concentrated on *Agrilus* spp. In China, no *S. agrili* were reared from six species of field-collected *Agrilus* larvae ($n$ = 2074). However, no-choice laboratory assays of some Chinese and North America *Agrilus* larvae showed some overlap in the physiological host range of *S. agrili*, although successful parasitism was lower in non-hosts than in EAB. Therefore, we evaluated the ecological host range of *S. agrili* using an olfactometer to determine the attractiveness of certain host plants: naïve mated *S. agrili* females were placed in vertical Y-tube olfactometers and given a choice of leaves and twigs from 14 tree species or clean air. We found *S. agrili* was only attracted to *Fraxinus pennsylvanica*, *F. velutina*, and the willow *Salix babylonica.* In nature, if parasitoids are not attracted to the host tree they will be unlikely to encounter and parasitize the non-target larvae. Given the combination of evidence from no-choice tests (lower parasitism rates or no attack on non-target species), olfactometer tests (only attracted to ash and willow), the lack of *S. agrili* reared from other *Agrilus spp.* in China, and that native *Spathius* rarely find and parasitize EAB, we predict incidental parasitism by *S. agrili* in non-target *Agrilus* spp.

## Conclusions

Considering the known adverse consequences of EAB on ash resources in North America, the lack of other options (except cutting down trees), the high potential benefit of these parasitoids, and the low potential risk to native *Agrilus* spp., we requested permission from the US Department of Agriculture early in 2007 to release the three parasitoids at selected sites in Michigan. After review by scientists and land managers at federal and state agencies, by university faculty members, and a 60-day public comment period, it was agreed that the potential benefits far outweighed the risks and release permits were issued. Field releases of *O. agrili* and *T. planipennisi* began in July 2007, and releases of *S. agrili* are expected to begin later in the summer or early fall.

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