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# HYMENOPTERAN PARASITOIDS ATTACKING THE INVASIVE EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE) IN WESTERN AND CENTRAL PENNSYLVANIA

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Abstract

We conducted field surveys of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, and associated larval parasitoids in western and central Pennsylvania (Cranberry Township in Butler County and Granville in Mifflin County) in the spring and fall of 2009. The survey procedure involved destructively debarking sections of the main trunk (bole) of EAB-infested green ash (*Fraxinus pennsylvanica* Marsh.) trees from the ground to the height of 2 m. Three species of the hymenopteran parasitoids were consistently recovered from EAB larvae observed in both survey sites, including two indigenous species of braconids, *Spathius laflammei* Provancher (= *Spathius benefactor* Matthews) and *Atanycolus nigropyga* Shenefelt and the exotic (accidentally introduced) eupelmid *Balcha indica* (Mani & Kaul). In addition, there are three unidentified species of hymenopteran parasitoids including two braconids *Atanycolus* sp. [possibly *Atanycolus disputabilis* (Cresson)] and *Spathius* sp. (at the Butler Co. site) and one ichneumonid *Dolichomitus* sp. (at the Mifflin Co. site). These parasitoids together parasitized 0.5-4.6% and 0.5-1.5% of the sampled EAB hosts at the Butler and Mifflin Co. sites, respectively. Parasitism rate by each species or group of those hymenopteran parasitoids varied between the two survey sites—with parasitism rates being generally higher at the Butler Co. site than at the Mifflin Co. site. Studies are needed to determine if those new associations of North American indigenous braconid parasitoids with EAB may play a complementary role in controlling this invasive pest.

Key Words: wood borers, *Agrilus planipennis*, *Spathius laflammei*, *Atanycolus nigropyga*, biological control

Resumen

Se realizaró un sondeo de campo sobre el barrenador esmeralda del fresno (BEF), *Agrilus planipennis* Fairmaire y parasitoides asociadas de las larvas en el oeste y centro de Pennsylvania (el municipio de Cranberry en el condado de Butler y Granville en el condado de Mifflin) en la primavera y el otoño del 2009. El procedimiento del sondeo consistió del descortezamiento destructivo de secciones del tronco principal de árboles de fresno (*Fraxinus pennsylvanica* Marsh.) infestado de BEF desde el suelo hasta la altura de 2 m. Tres especies de parasitoides del orden Hymenóptera fueron recuperadas regularmente de larvas de BEF observadas en los dos sitios de estudio, incluyendo 2 especies indígenas de bracónidos, *Spathius laflammei* Provancher (= *Spathius benefactor* Matthews) y *Atanycolus nigropyga* Shenefelt y lo exótico (introducido accidentalmente) eupelmido *Balcha indica* (Mani y Kaul). Además, hay 3 especies no identificadas de parasitoides del orden Hymenóptera de los cuales 2 especies de bracónidos, *Atanycolus*. [posiblemente *Atanycolus disputabilis* (Cresson)] y *Spathius* sp. (en el sitio en el condado de Butler), y una especie de ichneumónido, *Dolichomitus* sp. (en el sitio en el condado de Mifflin). Estos parasitoides juntos parasitaron 0.5 a 4.6% y del 0.5 - 1.5% de los BEF estudiadas en los sitios de los condados de Butler y Mifflin, respectivamente. La tasa de parasitismo por cada especie o grupo de los himenópteros parasitoides variaron entre los dos sitios de estudio - con la tasa de parasitismo generalmente más alto en el sitio en el condado de Butler que en el sitio en el condado de Mifflin. Se necesitan estudios para determinar si esas nuevas asociaciones de Norte América indígena parasitoides bracónido con BEF puede jugar un papel complementario en el control de esta plaga invasora.

Palabras Clave: barrenadores de madera, *Agrilus planipennis, Spathius laflammei, Atanycolus nigropyga*, control biológico

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleptera: Buprestidae), was first detected on North American ash (*Fraxinus* spp.) trees in South Detroit, Michigan in 2002 (Haack et al. 2002) (Fig. 1). A recent study of the dendrochronological evidence showed that *A. planipennis* was likely present (but had gone undetected) in Detroit, Michigan and Windsor, Ontario, Canada since the early to mid-1990s (Siegert et al. 2007). As of 2012, it has spread to 18 states in the northeastern and central U.S. and 2 provinces in Canada, and caused large-scale ash decline and mortality in many invaded-areas (EAB Information 2012). Besides the economic cost from treatment, removal, and replacement of infested landscape ash trees (Kovacs et al. 2010), this invasive pest has also had severe adverse effects on biodiversity and ecological services that depend exclusively on ash trees (Gandhi & Herms 2010).

Previous field studies conducted in the native range of EAB showed that several species of hymenopteran parasitoids were attacking immature stages (eggs and larvae) of EAB in Northeast China (Liu et al. 2007; Wang et al. 2007) and the Russian Far East (Duan 2012a). After safety-testing and regulatory approvals, 3 of these exotic parasitoid species, *Spathius agrili* Yang (Braconidae), *Tetrastichus planipennisi* Yang (Eulophidae), and *Oobius agrili* Zhang & Huang (Encyrtidae), were introduced from China to the U.S. for classical biocontrol of EAB (USDA APHIS 2007; Bauer et al. 2008; Duan et al. 2010, 2012b). Although these introduced parasitoids have established stable populations at several U.S. locations, parasitism rates of EAB by these introduced parasitoids are considerably lower in the introduced regions (1.5-5 % in Duan et al. 2010, 2012b) than rates (12-73%) reported from China (Liu et al. 2007, Wang et al. 2007) and Russia (Duan et al. 2012a). It remains to be seen if the classical biocontrol approach will result in successful control of EAB in the U.S.

In the meantime, field surveys conducted in North America have found several species of presumably native hymenopteran parasitoids associated with EAB larvae in Ontario (Lyons 2008), Michigan (Bauer et al. 2004, 2005; Cappaert & McCullough 2009; Duan et al. 2012b), Ohio (Kula et al. 2010), and Pennsylvania (Duan et al. 2009). These indigenous parasitoids consist of *Phasgonophora sulcata* Westwood (Chalcididae), as well as *Atanycolus cappaerti* Marsh and Strazanac, *Atanycolus disputabilis* Cresson, *Atanycolus hicoriae* Shenefelt, *Atanycolus simplex* Cresson, *Atanycolus tranquebaricae* Shenefelt, *Leluthia astigma* (Ashmead), and *Spathius floridanus* Ashmead (=*Spathius simillimus* Ashmead) (all Braconidae). Although parasitism rates of EAB larvae by most of the aforementioned parasitoids have been reportedly low (1-5%), *A. cappaerti* has been reported attacking >60% of EAB larvae in older infested ash stands in Michigan (Cappaert & McCullough 2009). In the present study, we report results from continued surveys of parasitoids in North America associated with EAB in western and central Pennsylvania, where the pest was discovered between 2007 and 2009. We also conducted laboratory tests with 2 recovered presumably native parasitoid species against EAB larvae to further confirm their associations and explore methods for their laboratory rearing.

## Materials and Methods

### Survey Sites

We conducted surveys in naturally occurring ash stands in forested areas in both western and central Pennsylvania in 2009 (Fig. 1). The western Pennsylvania site consisted of 2 small isolated forested areas (each ≈ 3 ha, approximately 2 km from each other) located in Cranberry Township (GPS Coordinates Decimal Degree: N 40.70667° W -80.09605°) in Butler County in the unglaciated Allegheny plateau, where an EAB population was first discovered in 2007. The central Pennsylvania site consisted of 2 nearby, larger forested areas (each ≈10 ha, approximately 5 km from each other) located at Granville (GPS Coordinate Decimal Degree: N 40.5441° W -77.6152°), Mifflin County, in the ridge and valley region, where an EAB population was first discovered in 2009. The Butler and Mifflin Co. sites, approximately 350 km apart, were primarily early successional, second-growth northern deciduous forest dominated by oak (*Quercus* spp.) and green ash (*Fraxinus pennsylvanica* Marshall). Less abundant trees species at these sites were shagbark hickory (*Carya ovata* (Mill.) K. Koch), boxelder (*Acer negundo* L.), black cherry (*Prunus serotina* Ehrh.), tulip poplar (*Liriodendtron tulipifera* L.), black walnut (*Juglans nigra* L.), cottonwood (*Populus deltoides* Bartr. ex Marsh.) and other poplars (*Populus* spp.), American basswood (*Tilia americana* L.), spruce (*Picea* spp.), and pine (*Pinus* spp.).

### Sampling Procedure

Surveys of parasitoids associated with EAB larvae were conducted in both the early spring (Feb-Jun) and fall (Sep and Oct) of 2009. At each survey time, we destructively sampled 2 to 4 green (*Fraxinus penssylvanica* Marsh.) ash trees with apparent symptoms of EAB infestation (e.g., bark splits, presence of woodpecker scaling and feeding damage, and epicormic growth on main trunk). All trees sampled were relatively large, with a mean (±SE) DBH (diameter at breast height) of 22.6 (±2.8) cm for the Butler Co. site and 27.2 (±1.9) cm for the Mifflin Co. site. For

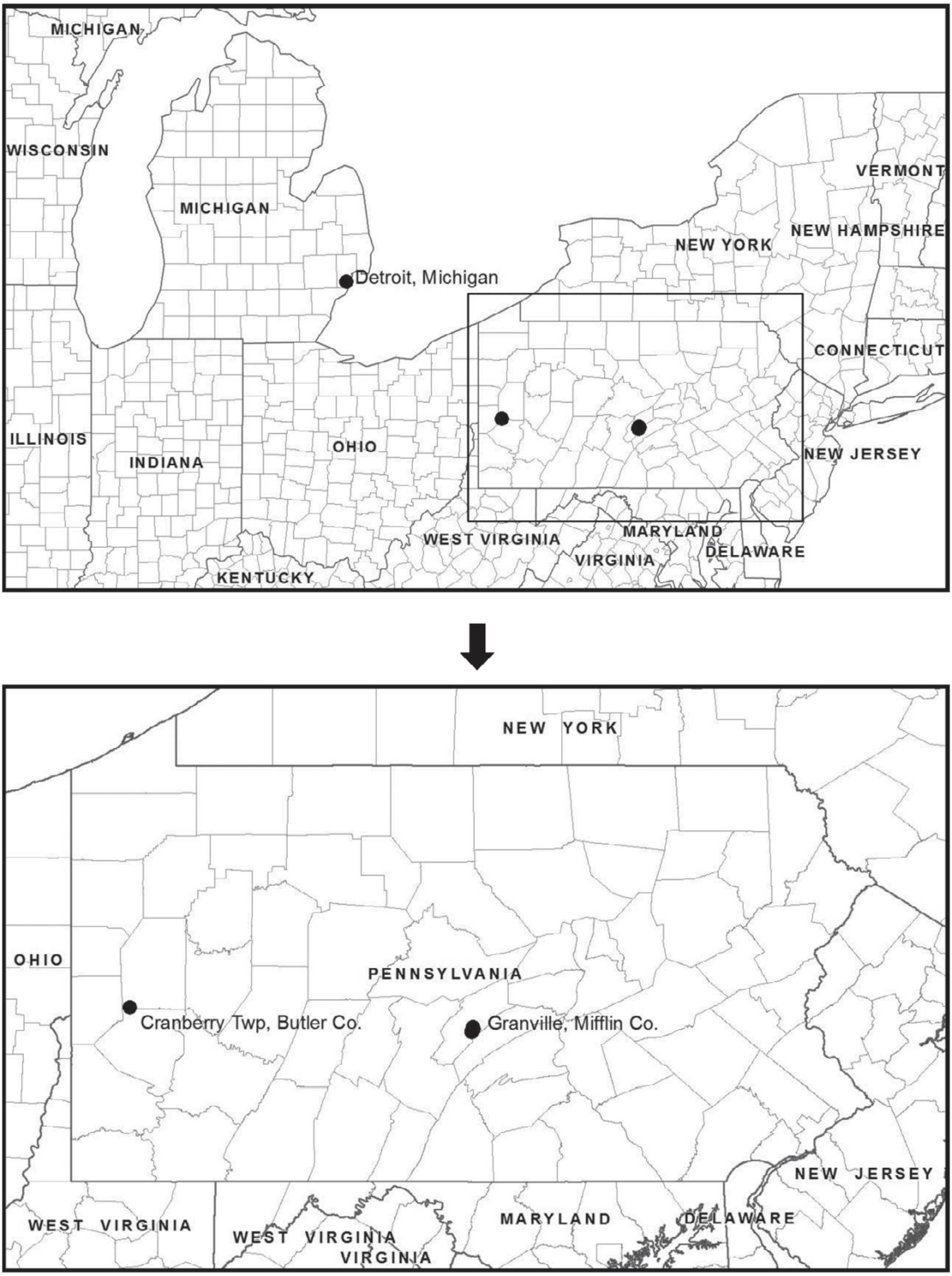


Fig 1. Locations where the emerald ash borer was first discovered and field survey samples were taken in western (Cranberry Township, Butler Co.) and central (Granville, Mifflin Co.), Pennsylvania.

sampling, the section of the main trunk (bole) of each ash tree from the ground to the height of 2 m was debarked with a draw knife and examined for presence of immature stages of EAB and associated parasitoids. No trees were felled in the field, instead a 1-m step ladder was used to assist in sampling the upper (1.5-2.0 m above the ground) section of the main trunk. To avoid damaging immature EAB under the bark, we first cut the bark using the draw knife to reach the cambium and the surface of the wood tissue and then peeled down the bark exposing the immature EAB and parasitoids in EAB galleries between the interface of cambium and sapwood. Mature larvae (J-shaped 4th instars), prepupae and/or pupae in the sapwood were sampled by removing the shallow (0.1-0.2 cm depth) sapwood tissues of their chamber with a chisel and hammer. In addition, mature larvae, prepupae and/or pupae nested in the utter bark were also sampled by breaking the bark into small pieces using a utility knife or hand. The exposed immature EAB stages (larvae, J-larvae, prepupae, and/or pupae) and associated parasitoids were collected with featherweight forceps (Bioquip Products #4750) and placed into individual cells of plastic culture plates (12 or 24 cells/plate) or Falcon Petri-Dishes (4-cm in diameter). All storage containers contained moist filter paper or Kimwipes to maintain high humidity. EAB larvae and associated parasitoids were returned to the USDA ARS Beneficial Insects Introduction Research Unit quarantine facility (BIIR, Newark, Delaware) to rear out parasitoid adults. Most of the parasitoids collected by debark sampling were immature stages (larvae and/or pupae), but a few were emerging adults. Adult parasitoids either directly collected from the field or recovered later from rearing in the laboratory were sent to the USDA ARS Systematic Entomology Laboratory (SEL, Beltsville, Maryland) or Paul Marsh (North Newton, Kansas) for identification. Voucher specimens were returned and deposited at the USDA ARS BIIR (Newark, Delaware). The fifth author (PMM) identified specimens of *Atanycolus* Förster, the fourth author (RRK) identified specimens of *Spathius* Nees, and Michael Gates (SEL) identified specimens of *Balcha indica* (Mani & Kaul) (Eupelmidae). The first author (JJD) identified the specimen of *Dolichomitus* sp (male) in comparison with the voucher specimen identified by RRK (Duan et al. 2009). EAB larvae were identified by JJD and RWF by comparing the specimens with ones reared from known EAB adults in the laboratory according to the characteristics described in Petrice et al. (2009) and Chamorro et al (2012).

### Laboratory Assay with Native Parasitoids

To evaluate parasitoid-host associations and explore for potential with laboratory rearing, we presented adults of *Spathius laflammei* Provancher (= *Spathius benefactor* Matthews) and *Atanycolus nigropyga* Shenefelt recovered from both survey sites to late instars of EAB larvae (3rd to 4th instars, including J-shaped mature 4th instar larvae) using the same method described in Duan & Oppel (2012). This method involved artificially inserting field-collected EAB larvae into ash sticks before exposing them to parasitoids. Sticks about 10 cm long and 2 to 5 cm in diam were freshly cut from field-grown green ash (*F. pennsylvanica*) trees. The sticks were sterilized by washing with warm soapy water and then placing them in a 10% bleach bath for ½ h after which they were flushed with copious amounts of cold tap water. The number of EAB larvae per stick varied according to the number of the parasitoids available for tests but were maintained approximately at a 2:1 host:parasitoid ratio in each assay. For the exposure assay, the EAB-infested ash sticks were exposed to adult parasitoids inside a test arena (17.6 × 12.6 × 10 cm ventilated polystyrene crisper boxes) for 2 wk. At the end of each assay, the exposed EAB-infested ash sticks were incubated in a growth chamber for a period of ≈30 days at 25 ± 2 °C, 55-65% RH, and 16:8 h L:D photoperiod for recovery of $F_1$ parasitoid progeny. After parasitoid emergence ceased, all sticks were dissected and parasitism of EAB larvae by the test parasitoids was scored based on the presence of parasitoid cocoons or small circular parasitoid exit holes (each ≈1-1.5 mm in diameter) on the stick associated with the gallery of each parasitized larva. Dead EAB larvae were also dissected under a stereomicroscope for any evidence (parasitoid cadavers) of unsuccessful parasitism. Percentage parasitism for each trial was calculated as proportion of immature EAB successfully attacked, as evidenced by the presence of parasitoid exit holes or progeny produced by the test parasitoid.

## Results and Discussion

At both the Butler and Mifflin Co. sites, 3 species of the hymenopteran parasitoids were consistently recovered from EAB larvae observed in the survey. Two of those species, *A. nigropyga* and *S. laflammei*, are presumably native to North America as they were originally collected and described as species in North America (Krombein et al. 1979; Marsh & Strazanac 2009). The other species is an accidentally introduced (exotic) eupelmid, *B. indica* (Gibson 2005). Two morphospecies of Braconidae, *Atanycolus* sp. poss. *disputabilis* (Cresson) and *Spathius* sp. (Butler Co. site), and one of Ichneumonidae, *Dolichomitus* sp. (Mifflin Co. site), were also reared but not identified to species. Rates of parasitism of EAB larvae by these parasitoids ranged from 0.5 to 4.6% at the Butler Co. site and from 0.5 to 1.5% at the Mifflin Co. site [Table 1]. Parasitism rates by those parasitoids appeared to have varied between the 2 survey sites, with parasitism rates generally higher at the Butler Co. site than at the Mifflin Co. site.

While *B. indica* inflicted the highest rate (4.6%) of parasitism of EAB larvae, prepupae, and pupae at the Cranberry site, *S. laflammei* caused the second highest rate (2.6%) of parasitism of EAB larvae (3rd to 4th instars) at the same site. *Spathius laflammei* caused the highest level of parasitism at the Granville site, with a rate of 1.05%. The parasitoids not identified to species together caused approximately 0.5% of EAB larval parasitism in both survey sites.

As reported previously (Duan et al. 2009), *B. indica* was observed in association with remains of EAB larvae (2nd to 4th instars), prepupae, and/or pupae. However, *A. nigropyga*, *A.* sp. poss. *disputabilis*, *S. laflammei*, and *Spathius* sp. were observed only in association with remains of 3rd to 4th instar larvae, not with prepupae and/or pupae. While *B. indica* and *Atanycolus* spp. were solitary in association with immature EAB stages, *Spathius spp.* were gregarious with a brood size ranging from 3 to 9 parasitoid larvae or cocoons in association with remains of each parasitized EAB larva. A recent laboratory study (Duan et al. 2011) showed that *B. indica* took a mean of 83 days (ranging from 47 to 129 days) to complete its life cycle at the constant 25 °C temperature. Based on this data, Duan et al (2011) suggested that *B. indica* might not have more than 2 generations in the mid-Atlantic and Midwest regions of United States, where normal growing seasons - with average temperature above 25 °C - normally occur less than 6 months (May-Oct). Currently, we have little knowledge about the life history of the 2 native braconids, *S. laflammei* and *A. nigropyga*, and do not know how many generations they can potentially have on emerald ash borer populations in these regions.

Our laboratory assays showed that field-recovered *S. laflammei* successfully attacked >50% of 3rd to 4th instars of EAB larvae ($N$ = 44) inserted into green ash sticks. A total of 121 $F_1$ parasitoid progeny (adults) were produced from 23 parasitized EAB larvae, with an average brood size (± SE) of 4.8 (±1.5) and female:male ratio of approximately 3:1 across different assays ($N$ = 5). In contrast, laboratory assays ($N$ = 5) with field-recovered *A. nigropyga* adults failed to produce parasitism of late instar EAB larvae (N = 15) inserted in green ash sticks. This indicates that the laboratory conditions used are unsuitable for rearing *A. nigropyga* on EAB larvae inserted into ash sticks. Although it is probable that *A. nigropyga* collected from our sampled ash trees might be associated with other native ash-boring buprestid beetle larvae, we did not observe any other wood-boring larvae other than EAB in our sampled trees.

TABLE 1. EMERALD ASH BORER LARVAE AND PARASITOIDS RECOVERED/COLLECTED FROM EAB-INFESTED ASH TREES (SPRING AND FALL, 2009).

| Sampling Sites | Number of Trees Sampled | Sampling Period | Total No. Viable EAB Larvae Observed | No. EAB Larvae Parasitized by *Spathius laflammei* (% Parasitism) | No. EAB Larvae Parasitized by *Atanycolus nigropyga* (% Parasitism) | No. EAB Larvae Parasitized by *Balcha indica* (% Parasitism) | No. EAB Larvae Parasitized by Other Parasitoids (% Parasitism)* |
|---|---|---|---|---|---|---|---|
| Cranberry | 11 | Feb-Apr; Sep-Oct | 390 | 10 (2.56%) | 4 (1.03%) | 18 (4.61%) | 2 (0.51%) |
| Granville | 19 | Mar-Jun; Sep-Oct | 378 | 4 (1.05%) | 1 (0.26%) | 2 (0.53%) | 2 (0.52%) |
| Pooled | 30 | | 768 | 14 (1.82%) | 5 (0.65%) | 20 (2.60%) | 4 (0.52%) |

*Including all the unidentified species (*Spathius* sp. *Atanycolus* sp., and *Dolichomitus* sp.)

EAB was first discovered in 2007 and 2009, respectively, in Cranberry Township (Butler Co.) and at Granville (Mifflin Co.) (Pennsylvania State University 2009). However, the extent of damage to ash trees in these 2 areas and the size of the infestation suggest that EAB might have been there several years earlier (JJD and RWF, unpublished data). Previous surveys conducted in 2008 in Cranberry Township (Butler Co.) only recovered 2 locally extant parasitoids, *B. indica* and *Eupelmus pini* Taylor (Eupelmidae), that caused about 3% parasitism of EAB larvae (Duan et al. 2009). The present survey demonstrated that 2 groups of presumably native braconid parasitoids, *Atanycolus* spp. and *Spathius* spp., are able to use EAB as a host and caused low level (< 3%) EAB parasitism in the 2 survey areas. As EAB becomes more firmly established with increased population densities in infested areas in the U.S., the role of parasitoids native to North America in suppressing EAB populations should be explored further.

Recent field studies conducted in Michigan, the epicenter of EAB infestation in the U.S., showed that presumably native parasitoids (i.e., *Atanycolus* spp., *P. sulcata*) inflicted relatively high rates (10-60%) of EAB parasitism (Cappaert et al. 2009; Duan et al. 2012b) in some older EAB-infested sites. In addition, Duan et al. (2012b) sampled the same sites repeatedly in Michigan over 2 yr and captured the succession and prevalence of parasitoid species attacking immature EAB at those sites. Such dynamic changes in the prevalence and species composition of EAB parasitoids will most likely continue in newly infested areas or regions and will vary with many ecological factors, such as tree species and *Agrilus* spp. from which these parasitoids originated, host range(s) of these parasitoids, local EAB density, and degree of disturbance due to ash mortality and decline. Along with the current EAB biocontrol program that involves release and establishment of introduced (exotic) parasitoids in the U.S., we recommend continuous study of indigenous parasitoids, such as *Atanycolus* spp. and *Spathius* spp., in North America for their complementary role in controlling EAB populations and/or their potential interactions with introduced biocontrol agents such as *S. agrili* and *T. planipennisi*. Future laboratory and/or field studies on the biology and life history of these native parasitoids would not only benefit in the method development for rearing them, but also in gaining insights into their potential for use in suppressing EAB populations.

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