Progress in the classical biological control of Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) in North America—CORRIGENDUM

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Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America

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Abstract—First detected in North America in 2002, the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae), an invasive phloem-feeding beetle from Asia, has killed tens of millions of ash (*Fraxinus* Linnaeus; Oleaceae) trees. Although few parasitoids attack EAB in North America, three parasitoid species were found attacking EAB in China: the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) and two larval parasitoids *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae). In 2007, classical biological control of EAB began in the United States of America after release of these three species was approved. In 2013, release of the larval parasitoids was approved in Canada. Research continues at study sites in Michigan, United States of America where the establishment, prevalence, and spread of *O. agrili* and *T. planipennisi* have been monitored since 2008. However, establishment of *S. agrili* remains unconfirmed in northern areas, and its release is now restricted to regions below the 40th parallel. In 2015, approval for release of *Spathius galinae* Belokobylskij (Hymenoptera: Braconidae), an EAB larval parasitoid from the Russian Far East, may be granted in the United States of America. Researchers are guardedly optimistic that a complex of introduced and native natural enemies will regulate EAB densities below a tolerance threshold for survival of ash species or genotypes in forested ecosystems.

Introduction

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive phloem-feeding beetle from Asia that attacks and kills ash trees (*Fraxinus* Linnaeus; Oleaceae) in North America. First discovered in Michigan, United States of America and nearby Ontario, Canada in 2002, EAB was accidentally introduced from China during the 1990s, presumably in solid-wood packaging materials (Haack *et al.* 2002; Cappaert *et al.* 2005; Bray *et al.* 2011; Keever *et al.* 2013; Siegert *et al.* 2014). Soon after the discovery of this invasive pest, regulatory agencies began surveying for EAB infestations, establishing quarantines, and initiating eradication activities (Federal Register 2003; Poland and McCullough 2006; Herms and McCullough 2014). However, efforts to eradicate EAB were eventually abandoned due to limited efficacy of EAB-surveillance tools, long-distance human-mediated movement of EAB, and natural dispersal of EAB through flight (Federal Register 2003; Cappaert *et al.* 2005; Government Accounting Office 2006; Taylor *et al.* 2010; Canadian Food Inspection Agency 2014; United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) 2014). As of February 2015, EAB is known to infest trees in 25 states in the United States of America (Arkansas, Colorado, Connecticut,

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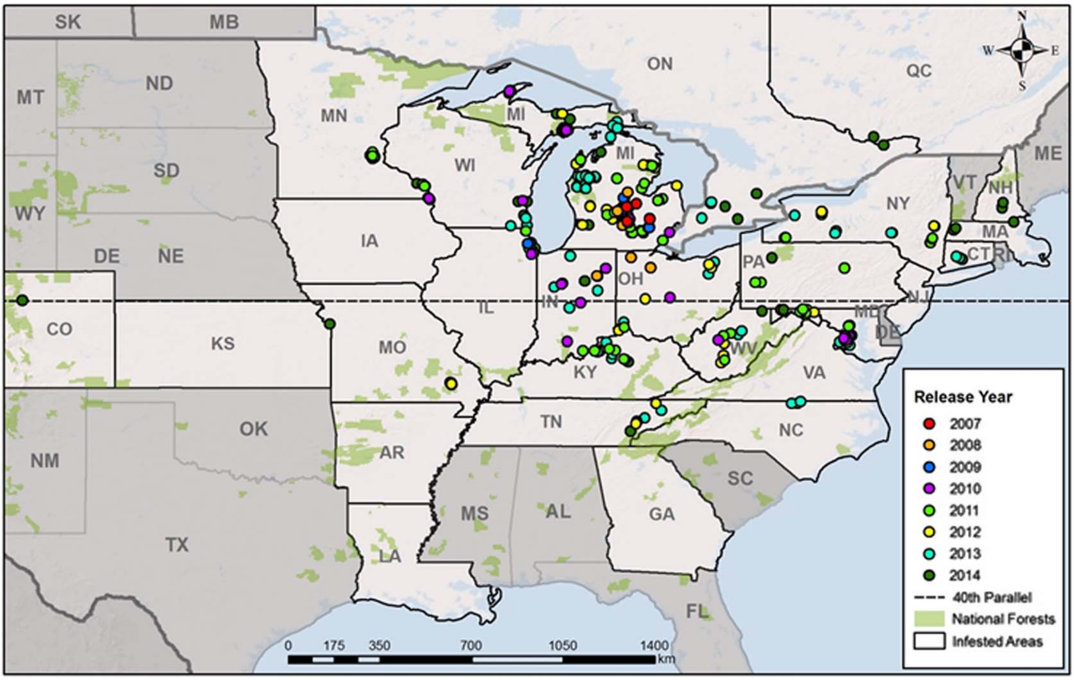
Fig. 1. Known distribution of emerald ash borer, *Agrilus planipennis*, in North America as of February 2015, and the locations of EAB biocontrol agents, *Oobius agrili*, *Tetrastichus planipennisi*, and *Spathius agrili*, introduced to North America from China from 2007 to 2014. Service layer credits: United States National Park Service. Data sources: United States Department of Agriculture, Animal and Plant Health Inspection Service; Canadian Food Inspection Agency; and www.mapbiocontrol.org. Map created by Applied Spatial Ecology and Technical Services, Department of Entomology, Michigan State University (East Lansing, Michigan, United States of America).



Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, and Wisconsin) and two Canadian provinces (Ontario and Québec) (Fig. 1).

Natural enemy surveys of EAB populations are conducted to understand the population dynamics of EAB in North America. Although a variety of native mortality factors, including woodpeckers, insect predators, hymenopteran parasitoids, and fungal pathogens have been found to prey on EAB at high densities, their impacts are lower than that experienced by native *Agrilus* Curtis species and insufficient to regulate EAB in indigenous ash species (Loerch and Cameron 1983; Lindell *et al.* 2008; Bauer *et al.* 2008, 2014; Cappaert and McCullough 2009; Duan *et al.* 2009, 2010, 2012a, 2013b, 2014a; Jennings *et al.* 2013; Bauer *et al.* 2014). In Asia, where EAB, insect natural enemies, and Asian ash species co-evolved, however, researchers are finding host-specific parasitoids that are capable of suppressing EAB populations to tolerable levels (Liu *et al.* 2003, 2007; Bauer *et al.* 2008; Rebek *et al.* 2008; Duan *et al.* 2012c).

Classical biological control, a long-range and sustainable approach to pest management, involves the introduction and establishment of natural enemies from the native range of an invasive pest. Before initiating a classical biological control programme, however, researchers compare mortality factors of the pest in newly invaded areas to regions where it originated. Herein, we provide an overview of the developing classical biological control programme for EAB that began with the first introductions of EAB parasitoids from China to the United States of America in 2007 (Federal Register 2007;

Bauer *et al.* 2008, 2014, 2015) and Canada in 2013 (Canadian Food Inspection Agency 2014).

Natural enemies of EAB in North America

Soon after EAB was discovered in North America in 2002, natural enemy surveys began in Michigan, Ontario, and other regions. Woodpeckers, which forage on immature wood-boring insects under tree bark, are the dominant natural enemy of EAB throughout infested areas of North America. For example, woodpecker predation on individual trees, accounted for up to 95% EAB larval mortality (Cappaert *et al.* 2005), although mortality rates generally averaged 35–40% (Anulewicz *et al.* 2007; Lindell *et al.* 2008; Duan *et al.* 2012a, 2014a; Jennings *et al.* 2013). In a separate study of bird populations in the vicinity of older EAB infestations in the United States of America, Koenig *et al.* (2013) reported higher populations of certain woodpecker and other bird species following the EAB invasion. A diversity of entomopathogenic fungi are also found as natural enemies of EAB larvae, with an average 2% rate of infection at field sites in southeast Michigan (Liu and Bauer 2006; Castrillo *et al.* 2010).

Field studies of native insect natural enemies attacking EAB larvae in the United States of America and Canada reveal a diversity of hymenopteran parasitoids; however, their combined prevalence is generally low (< 1–5%), and no native egg parasitoids have been reported (Table 1) (Bauer *et al.* 2015). These parasitic Hymenoptera are more specific to niche than to host, generally attacking larvae of the larger species of *Agrilus* (Taylor *et al.* 2012; Bauer *et al.* 2014). Much of the research on EAB natural enemies has been done in Michigan (Cappaert and McCullough 2009; Tluczek *et al.* 2010; Bauer *et al.* 2014) although similar work has been done in Pennsylvania (Duan *et al.* 2009; 2013b), Ohio (Kula *et al.* 2010), and Ontario (Roscoe 2014). In southeast Michigan, several species of predaceous Coleoptera in the genera *Enoclerus* Gahan (Coleoptera: Cleridae), *Catogenus* Westwood (Passandridae), and *Tenebroides* Latreille (Coleoptera: Trogossitidae) were found under the bark of ash trees heavily infested by EAB; the larvae and adults of these coleopteran predators were confirmed to prey on EAB larvae in the laboratory (Bauer *et al.* 2004).

Of the native parasitic Hymenoptera attacking EAB larvae in North America (Table 1), solitary ectoparasitoids in the genus *Atanycolus* Förster (Hymenoptera: Braconidae) and the solitary endoparasitoid *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), are the most common (Bauer *et al.* 2008, 2015; Duan *et al.* 2009, 2013b). Some species of *Atanycolus* and *P. sulcata*, however, are responding numerically to increasing EAB populations at some sites in Michigan (Cappaert and McCullough 2009; Duan *et al.* 2014a). Other less common larval parasitoids include gregarious ectoparasitoids in the genus *Spathius* Nees (Hymenoptera: Braconidae) and several solitary ectoparasitoid species including *Leluthia astigma* (Ashmead) (Hymenoptera: Braconidae) (Kula *et al.* 2010) and species of *Eupelmus* Dalman (Hymenoptera: Eupelmidae), *Eurytoma* Illiger (Hymenoptera: Eurytomidae), and *Dolichomitus* Smith (Hymenoptera: Ichneumonidae) (Duan *et al.* 2009, 2013b). Emerald ash borer larvae are also parasitised sporadically by the solitary larval ectoparasitoid *Balcha indica* (Mani and Kaul) (Hymenoptera: Eupelmidae), which was inadvertently introduced to North America from southeast Asia. This exotic parasitoid is now naturalised in the eastern United States of America and attacks the larvae of several woodboring Coleoptera (Bauer *et al.* 2004; Gibson 2005; Duan *et al.* 2009, 2011c, 2014a).

Natural history of key EAB parasitoids native to North America

Five species of native braconids in the genus *Atanycolus* are known to parasitise EAB larvae (Bauer *et al.* 2004, 2008, 2014, 2015; Duan *et al.* 2009, 2010, 2012a, 2013b, 2014a; Cappaert and McCullough 2009; Marsh *et al.* 2009) (Table 1). These relatively large wasps are solitary, ectoparasitic idiobionts of late-instar *Agrilus* larvae, with one or two generations per year. The natural history of *A. cappaerti* Marsh and *A. hicoriae* Shenefelt are synchronised with the one-year or two-year life cycle of EAB in Michigan (Cappaert and McCullough 2009; Marsh *et al.* 2009). Although parasitism of EAB larvae by native parasitoids is generally low, *A. cappaerti* proved to be capable of significant parasitism in some heavily infested ash trees in Michigan; it also parasitises

Table 1. Indigenous or naturalised parasitic Hymenoptera known to attack emerald ash borer, *Agrilus planipennis*, larvae in North America before introduction of the three classical biological control agents from China.

Family	Parasitoid natural history	Species	Citations
Braconidae	Solitary larval ectoparasitoid	*Atanycolus cappaerti* Marsh and Strazanac	Cappaert and McCullough (2009), Marsh *et al.* (2009), Duan *et al.* (2012a), Bauer *et al.* (2015)
Braconidae	Solitary larval ectoparasitoid	*Atanycolus disputabilis* (Cresson)	Duan *et al.* (2013b)
Braconidae	Solitary larval ectoparasitoid	*Atanycolus hicoriae* Shenefelt	Bauer *et al.* (2008, 2015), Cappaert and McCullough (2009), Duan *et al.* (2009, 2013b)
Braconidae	Solitary larval ectoparasitoid	*Atanycolus nigropopyga* Shenefelt	Duan *et al.* (2013b)
Braconidae	Solitary larval ectoparasitoid	*Atanycolus simplex* Cresson	Bauer *et al.* (2008), Duan *et al.* (2009)
Braconidae	Solitary larval ectoparasitoid	*Leluthia astigmata* (Ashmead)	Kula *et al.* (2010)
Braconidae	Gregarious larval ectoparasitoid	*Spathius floridanus* Ashmead* (= *S. simillimus* Ashmead)*	Bauer *et al.* (2004), Marsh and Strazanc (2009), Duan *et al.* (2013b), Bauer *et al.* (2015)
Braconidae	Gregarious larval ectoparasitoid	*Spathius laflammei* Provancher (= *S. benefactor* Matthews)	Duan *et al.* (2013b)
Chalcididae	Solitary larval endoparasitoid	*Phasgonophora sulcata* Westwood	Bauer *et al.* (2004), Duan *et al.* (2010, 2014a), Roscoe (2014), Bauer *et al.* (2015)
Eupelmidae	Solitary larval ectoparasitoid, thelytokous parthenogenesis	*Balcha indica* (Mani and Kaul)≅	Bauer *et al.* (2004, 2008), Gibson (2005), Duan *et al.* (2009, 2011b), Duan *et al.* (2014a)
Eupelmidae	Solitary larval ectoparasitoid	*Eupelmus* Dalman species	Bauer *et al.* (2004), Duan *et al.* (2009, 2013b)
Ichneumonidae	Solitary larval ectoparasitoid	*Cubocephalus* Ratzeburg species	Duan *et al.* (2009, 2013b)
Ichneumonidae	Solitary larval ectoparasitoid	*Dolichomitus* Smith species	Duan *et al.* (2009, 2013b)
Ichneumonidae	Solitary larval ectoparasitoid	*Orthizema* (Förster) species	Duan *et al.* (2009, 2013b)

* Differences in natural history and genetics indicate that *S. floridanus* and *S. simillimus* are distinct species (Bauer *et al.* 2015).
≅ Inadvertently introduced from southeast Asia and naturalised in the eastern United States of America.

native *Agrilus* species in other host trees (Cappaert and McCullough 2009).

Another increasingly important native parasitoid of EAB larvae in eastern North America is *P. sulcata*, a solitary, endoparasitic koinobiont also known to parasitise the larvae of native *Agrilus* species (Barter 1957, 1965; Côté and Allen 1980; Haack *et al.* 1981; Bauer *et al.* 2004, 2014, 2015; Duan *et al.* 2012a, 2014a; Roscoe 2014). In Michigan, *P. sulcata* adults oviposit in young EAB larvae in July and August, their larvae develop in the posterior hemocoel of EAB larvae, and pupation occurs after one or two years during the host prepupal period (L.S.B. and J.J.D., personal observation).

Insect natural enemies of EAB in Eurasia

Native range of EAB in Asia

Less than a decade before EAB was discovered in North America, *Agrilus planipennis* (type locality in China) was synonymised with *Agrilus feretrius* Obenberger (type locality in Taiwan), *Agrilus marcopoli* Obenberger (type locality in Mongolia), and *Agrilus marcopoli ulmi* Kurosawa (type locality in Japan), leading to a known native range extending throughout China, Japan, Korea, Mongolia, the Russian Far-East, and Taiwan (Yu 1992; Jendek 1994; Jendek and Grebennikov 2011) (Fig. 2). Although *Fraxinus* species were the only larval hosts reported for *A. planipennis* in China (Yu 1992; Liu *et al.* 2003; Zhao *et al.* 2005), larval host records for *A. marcopoli* and *A. marcopoli ulmi* in Korea and Japan included *Juglans*, *Pterocarya*, and *Ulmus* (Ko 1969; Akiyama and Ohmomo 1997).

At that time, only limited literature on EAB was available, mainly from China, where EAB was considered a minor and periodic pest of native *Fraxinus chinensis* Roxburgh and *F. mandshurica* Ruprecht (Yu 1992; Liu *et al.* 1996). However, there were also reports of EAB outbreaks in *F. velutina* Torrey, an ash species native to North America and widely planted for landscape in the region of Tianjin, China (Zhang *et al.* 1995; Liu *et al.* 1996). Emerald ash borer was also known from the provinces of Hebei, Heilongjiang, Inner Mongolia, Jilin, Liaoning, and Shandong, China (Chinese Academy of Science 1986); more recently, EAB was reported from Sichuan province (Jendek and Grebennikov 2011) (Fig. 2).

Foreign exploration for EAB natural enemies in Asia

In 2003, foreign exploration for insect natural enemies of EAB began in China when researchers began surveying ash trees in the provinces of Hebei, Heilongjiang, Jilin, Liaoning, Tianjin, and Shandong, and the cities of Beijing and Tianjin (Liu *et al.* 2003) (Fig. 2). During this survey, EAB infestations were found in each region except Shandong, and higher EAB densities were consistently found in North American ash species growing in China than in native Asian species (Zhao *et al.* 2005). Additional foreign exploration for EAB natural enemies was done in areas of South Korea (Williams *et al.* 2005, 2006, 2010), the Russian Far East (Williams *et al.* 2010; Duan *et al.* 2012c), and more recently in European Russia, subsequent to EAB's discovery near Moscow, Russia (Orlova-Bienkowskaja and Belokobylskij 2014) (Table 2).

Insect natural enemies of EAB in Asia

After a decade of field research in northeast China (2003–2013), the dominant insect natural enemies of EAB discovered were the larval parasitoid *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Table 2). These two species were reared from EAB-infested Asian and North American ash trees in Beijing, and Hebei, Heilongjiang, Jilin, Liaoning provinces (Liu *et al.* 2003; Zhang *et al.* 2005; Yang *et al.* 2006; Bauer *et al.* 2014, 2015; L.S.B. and J.J.D., personal observation) (Fig. 2). In a study of EAB population dynamics (2004–2005) in Jilin province, Liu *et al.* (2007) reported the phenology of *T. planipennisi* and *O. agrili* to be closely synchronised with the life cycle of EAB and estimated that the combined larval and egg parasitism reduced EAB densities by 74%. The only other egg parasitoid reared from EAB eggs was an undescribed species of *Ooencyrtus* Ashmead (Hymenoptera: Encyrtidae) (a single adult female) from Jilin province; EAB egg parasitism was confirmed in the laboratory with the successful emergence of an adult male (L.S.B., personal observation).

In the port city of Tianjin southeast of Beijing, the larval parasitoid *Spathius agrili* Yang

Fig. 2. Known native range of emerald ash borer, *Agrilus planipennis*, in Asia and other regions where foreign exploration for EAB natural enemies has occurred since 2003. Service layer credits: United States National Park Service. Data source: https://sites.google.com/site/eduardjendek/world-distribution-of-agrilus-planipennis. Map created by United States Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry, Office of Knowledge Management (Durham, New Hampshire, United States of America).

(Hymenoptera: Braconidae) attacks EAB infesting North American *F. velutina* planted as plantation and landscape trees, with an average parasitism rates of 30–50% (Liu *et al.* 2003; Xu 2003; Yang *et al.* 2005) (Fig. 2). Also in Tianjin, a generalist parasitoid of woodborer larvae and pupae, *Sclerodermus pupariae* Yang and Yao (Hymenoptera: Bethylidae), parasitises up to 13% of EAB (Yang *et al.* 2012).

In the Russian Far East in the region of Primorsky near Vladivostok, Duan *et al.* (2012a) reported three species of larval parasitoids: *T. planipennisi*, *Spathius galinae* Belokobylskij (Hymenoptera: Braconidae) (Belokobylskij *et al.* 2012), and *Atanycolus nigriventris* Vojnovskaja-Krieger (Hymenoptera: Braconidae) and an egg parasitoid, undescribed in the genus *Oobius* Trjapitzin (Hymenoptera: Encyrtidae) (Fig. 2)

Table 2. Parasitic Hymenoptera attacking emerald ash borer, *Agrilus planipennis*, in Asia and Europe, and classical biological control agents in North America.

Family	Parasitoid natural history	Species	Native range	Citations
Bethylidae	Gregarious larval ectoparasitoid	*Sclerodermus pupariae* Yang and Yao	China	Wu *et al.* (2008), Wang *et al.* (2010), Tang *et al.* (2012), Yang *et al.* (2012)
Braconidae	Solitary larval ectoparasitoid	*Atanycolus nigriventris* Vojnovskaja-Krieger	Russian Far East	Williams *et al.* (2010), Belokobylskij *et al.* (2012), Duan *et al.* (2012a)
Braconidae	Gregarious larval ectoparasitoid	*Spathius agrili* Yang[*,≅]	China	Liu *et al.* (2003), Xu (2003), Yang *et al.* (2005), Wang *et al.* (2008, 2010), Ulyshen *et al.* (2010b), Bauer *et al.* (2015)
Braconidae	Gregarious larval ectoparasitoid	*Spathius galinae* Belokobylskij[⊥]	Russian Far East	Williams *et al.* (2010), Belokobylskij *et al.* (2012), Duan *et al.* (2012c)
Braconidae	Gregarious larval ectoparasitoid	*Spathius polonicus* Niezabitowski	Europe	Orlova-Bienkowskaja and Belokobylskij (2014)
Encyrtidae	Solitary egg parasitoid, thelytokous parthenogenesis	*Oobius agrili* Zhang and Huang[*,§]	China	Zhang *et al.* (2005), Liu *et al.* (2007), Abell *et al.* (2014), Bauer *et al.* (in press)
Encyrtidae	Solitary egg parasitoid, thelytokous parthenogenesis	*Oobius* Trjapitzin species	Russian Far East	J.J.D. (personal observation)
Encyrtidae	Solitary egg parasitoid	*Ooencyrtus* Ashmead species	China	L.S.B. (personal observation)
Eulophidae	Gregarious larval endoparasitoid	*Tetrastichus planipennisi* Yang[*,≅,§]	China, Russian Far East	Liu *et al.* (2003, 2007), Yang *et al.* (2006), Ulyshen *et al.* (2010a, 2010b), Duan *et al.* (2013a), Bauer *et al.* (2015)
Eulophidae	Gregarious larval endoparasitoid	*Tetrastichus telon* (Graham)[‖]	South Korea	Williams *et al.* (2010)

[*] Releases began for EAB biocontrol in the United States of America in 2007.
[≅] Approved for EAB biocontrol in Canada in 2013; only *T. planipennisi* is being released in northern regions.
[⊥] Pending approval in the United States of America, releases for EAB biocontrol may begin in 2015
[§] Establishment and spread is confirmed in the United States of America.
[‖] Tentative identification.

(Table 2). *Spathius galinae* and a species of *Tetrastichus,* tentatively identified as *T. telon* (Graham), were also reported attacking EAB in South Korea (Williams *et al.* 2010). In European Russia, near Moscow another larval parasitoid, *Spathius polonicus* Niezabitowski (Hymenoptera: Braconidae) known to attack woodborers in Europe, was recently discovered parasitising EAB (Orlova-Bienkowskaja and Belokobylskij 2014).

Natural history and host range studies of key EAB biocontrol agents

Tetrastichus planipennisi. A gregarious endoparasitic koinobiont of EAB larvae, *T. planipennisi* was discovered in 2003 during the initial foreign exploration for EAB and its natural enemies in northeast China (Liu *et al.* 2003; Yang *et al.* 2006). In Jilin province, the adults begin emerging in May, up to four generations may be completed during each year, and mature larvae or prepupae overwinter in host galleries (Liu *et al.* 2007; Ulyshen *et al.* 2010a; Duan *et al.* 2011b; Bauer *et al.* 2015).

Following importation of *T. planipennisi* from Jilin province to the United States Department of Agriculture Forest Service quarantine laboratory in East Lansing, Michigan, a breeding colony was developed in EAB larvae. The host specificity of *T. planipennisi* was evaluated with paired no-choice assays using EAB larvae as positive controls (Badendreier *et al.* 2005; Federal Register 2007; Bauer *et al.* 2014). The larvae of 14 species of wood-boring insects, including eight species of Buprestidae (Coleoptera) (five of *Agrilus* Curtis and three of *Chrysobothris* Eschscholtz), five species of Cerambycidae (Coleoptera) (one each of *Neoclytus* Thomson*, Megacyllene* Casey, *Astylopsis* Casey*, Monochamus* Megerle, and an unknown cerambycid from apple), and one species of wood-boring sawfly (*Janus abbreviates* (Say); Hymenoptera: Cephidae), were tested in their respective host plants. Larvae of non-wood-boring insects were also tested after they were inserted into ash branches, including one beetle in the family Tenebrionidae (Coleoptera) and two moths, one each in Pyralidae and Sphinghidae (Lepidoptera). Overall, *T. planipennisi* parasitised only the larvae of EAB. After careful risk-benefit analyses in 2006, the North American Plant Protection Organization (NAPPO) approved the release of *T. planipennisi* as an EAB biocontrol agent in North America, and in 2007, United States Department of Agriculture, APHIS issued permits for its environmental release in Michigan (Federal Register 2007; Bauer *et al.* 2008, 2014, 2015).

Oobius agrili. A solitary egg parasitoid, *O. agrili* reproduces through thelytokous parthenogenesis and was first reared from EAB eggs collected in Jilin province, China in 2004 (Zhang *et al.* 2005). In this region of China, *O. agrili* adults emerge from parasitised EAB eggs in late June (Liu *et al.* 2007). After emergence, these tiny wasps parasitise EAB eggs laid on the bark of ash trees. The first generation of *O. agrili* takes about a month, and wasps complete one or more generations during summer and early fall, when EAB egg parasitism peaks. This parasitoid spends the winter as diapausing mature larvae or prepupae inside EAB eggs until the following June when EAB oviposition begins (Liu *et al.* 2007; Bauer *et al.* 2015).

Eggs of EAB from Jilin province, some parasitised with *O. agrili*, were imported to the United States Department of Agriculture, Forest Service quarantine laboratory in Michigan from 2004 to 2009. These were used to develop colonies of *O. agrili* using laboratory-reared EAB eggs. Although males were occasionally reared from EAB eggs collected in China, only females were used to establish the laboratory colonies. Host specificity was tested by exposing the eggs of other insect species, on branches of their respective host trees, to adult *O. agrili* in no-choice assays and paired-choice assays, with EAB eggs on ash used as positive controls (Badendreier *et al.* 2005; Federal Register 2007; Bauer *et al.* 2014). Six species of buprestids in the genus *Agrilus* and two species of cerambycids were reared in the laboratory and eggs, laid on their respective hosts, were exposed to adult *O. agrili*. Similarly exposed were the eggs of four Lepidoptera species that had been laid on ash branches from the following families: Bombycidae, Pieridae, Sphingidae, and Tortricidae. In no-choice tests, researchers found *O. agrili* parasitised only eggs of the three largest *Agrilus* species (*A. anxius* Gory, *A. bilineatus* (Weber), and *A. ruficollis* (Fabricius)), each with eggs similar in size to EAB eggs. To further evaluate parasitoid host preferences, paired-choice assays were conducted by exposing *O. agrili* adults to the eggs of EAB on ash and the eggs of the above mentioned species on their respective hosts (*A. anxius* on *Betula pendula* Roth (Betulaceae), *A. bilineatus* on *Quercus rubra* Linnaeus (Fagaceae), or *A. ruficollis* on *Rubus* Linnaeus (Rosaceae)). In paired choice tests, *O. agrili* showed a consistent preference for EAB eggs on ash versus the eggs of these three *Agrilus* species on their host plants. Due to the known risk of EAB to ash in North America, NAPPO also approved the release of *O. agrili* as an EAB biocontrol agent in 2006, and United States Department of Agriculture, APHIS issued permits for its release to start in Michigan in 2007 (Federal Register 2007; Bauer *et al.* 2008, 2014, 2015).

Spathius agrili. *Spathius agrili*, a gregarious ectoparasitic idiobiont of late-instar EAB larvae, was the most abundant natural enemy of EAB found in the region of Tianjin, China (Liu *et al.* 2003; Xu 2003; Yang *et al.* 2005). It overwinters as mature larvae or prepupae inside silken cocoons in EAB host galleries, emerges in July and August with a female:male sex ratio of 4:1, and completes one or two generations per year in this region of China (Wang *et al.* 2006, 2008; Gould *et al.* 2011; Bauer *et al.* 2015).

Laboratory colonies of *S. agrili* from Tianjin were reared in EAB larvae at the Chinese Academy of Forestry in Beijing China and in a quarantine laboratory at the United States Department of Agriculture, APHIS laboratory in Buzzards Bay, Massachusetts (Wang *et al.* 2008; Yang *et al.* 2008; Gould *et al.* 2011). At both laboratories, similar no-choice tests of *S. agrili* host specificity were used to compare parasitism of EAB larvae inserted in ash branches to larval parasitism of other insect species inserted in their respective host plants (Yang *et al.* 2008). Nine *Agrilus* species, three from the United States of America (*A. anxius*, *A. bilineatus*, and *A. ruficollis*) and six from China were tested. Species of buprestids, one cerambycid, and one Curculionidae from China and six Lepidoptera larvae (in Pyralidae, Cossidae, and Carposinidae) were also tested. Although *S. agrili* successfully parasitised the larvae of six of the nine *Agrilus* species (*A. anxius*, *A. bilineatus*, *A. inamoenus* Kerremans, *A. mali* Matsumura, *A. zanthoxylumi* Hou), rates of larval parasitism by *S. agrili* was significantly lower in these non-target species than in EAB (Yang *et al.* 2008). Yang *et al.* (2008) also studied the behavioural responses of *S. agrili* females to the leaf volatiles from 14 species of woody plants growing in China using a Y-tube olfactometer. Foliage from the plant families Oleaceae (to which ash belongs), Rutaceae, Rosaceae, Salicaceae, Celastraceae, Juglandaceae, Leguminosae, and Simaroubaceae were tested, and only the foliage of *F. velutina* (Oleaceae), *F. pennsylvanica* (Oleaceae), *Prunus persica* Linnaeus (Rosacea), and *Ailanthus altissima* (Miller) (Simaroubaceae) were attractive to *S. agrili.* The attraction of *S. agrili* females to ash leaf volatiles was also demonstrated in a more recent study (Johnson *et al.* 2014). The results of these and other studies supported the view that EAB in ash is the primary host of *S. agrili*. In 2006, NAPPO also approved the release of *S. agrili* as an EAB biocontrol agent in North America, and USDA APHIS issued permits for its release in Michigan in 2007 (Federal Register 2007; Bauer *et al.* 2008, 2014, 2015).

Spathius galinae. *Spathius galinae*, a gregarious ectoparasitic idiobiont of late-instar EAB larvae (Belokobylskij *et al.* 2012), is known from the Russian Far East and South Korea near Daejeon (Williams *et al.* 2010; Duan *et al.* 2012c). In the Vladivostok region, mature *S. galinae* larvae or prepupae diapause inside cocoons in host galleries, emerge in early spring as adults, and complete two to three generations per year (Belokobylskij *et al.* 2012; Duan *et al.* 2012c).

Host range studies were completed after importation of *S. galinae* in 2010 to the United States Department of Agriculture, Agricultural Research Service quarantine laboratory in Newark, Delaware, where a colony was developed in EAB larvae reared in ash bolts (Duan *et al.* 2012c, 2014b). Using choice and no-choice assays, the host specificity of *S. galinae* was evaluated by United States Department of Agriculture researchers at quarantine laboratories in Delaware and Massachusetts (J.J.D. and J.R.G., personal observation). The larvae of 15 wood-boring insect species from North America were tested: 13 wood-boring beetles, one clearwing moth (Lepidoptera: Sesiidae), and one sawfly (Hymenoptera: Cephidae). Of the beetles, five were in *Agrilus* and another in the same family (Buprestidae). Researchers found that *S. galinae* parasitised the larvae of both EAB (in ash) and the larvae of gold-spotted oak borer, *Agrilus* *auroguttatus* Schaeffer (in oak), an invasive woodborer of oaks in California. However, parasitism rates were lower in *A. auroguttatus* (41%) than in EAB (71%). In 2013, NAPPO received and approved a petition to release *S. galinae* for EAB biocontrol in North America. In 2015, United States Department of Agriculture, APHIS posted an environmental assessment on the federal register for public comment on the release of *S. galinae* in the United States of America (Federal Register 2015). If a finding of no significant impact is determined, release permits will be issued in certain states for the 2015 field season.

EAB classical biological control

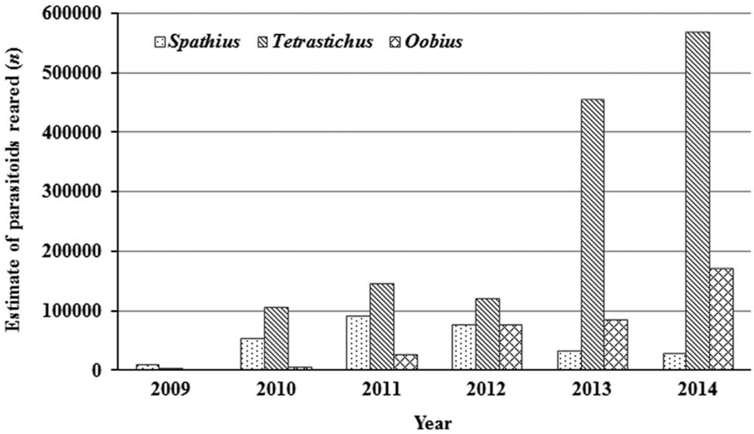
Parasitoid releases

After release permits were issued in July 2007 for *O. agrili*, *T. planipennisi*, and *S. agrili* in Michigan, adult wasps were released onto infested ash trees at peak EAB densities in seven different field sites during late summer and fall; from 2008 to 2009, similar release sites were started in Illinois, Indiana, Maryland, Ohio, and other areas of Michigan (Bauer *et al.* 2008) (Fig. 1). All sites are natural forested ecosystems, and although comparatively few parasitoids were released these first few years, parasitoid-recovery efforts over the following years indicated parasitoid establishment was successful at several research sites (Duan *et al.* 2013a; Abell *et al.* 2014). These early successes led to the development of a United States Department of Agriculture EAB Biological Control Program and construction of the APHIS EAB Biocontrol Facility in Brighton, Michigan (United States Department of Agriculture 2009). To provide guidance for land managers and regulatory agencies interested in managing EAB using classical biocontrol, researchers prepared a detailed guideline, which is updated periodically as new information and methods become available (United States Department of Agriculture 2013). To track, monitor, and map parasitoid releases, recoveries, and spread, researchers at Michigan State University and United States Department of Agriculture developed an online geospatial database known as MapBioControl.org (MapBioControl 2014).

In the EAB biocontrol guidelines (United States Department of Agriculture 2013), we recommend locating parasitoid-release sites in forested areas, woodlots, wooded wetlands, and riparian zones of at least 40 acres where the removal of declining ash is unlikely, with low to moderate EAB densities, at least 25% ash with a range of ash size classes, and interconnected with other forested ecosystems via ash corridors (*e.g.*, rivers, ditches), which facilitate parasitoid dispersal. Although most of the larger ash trees die off within a few years of release, they provide abundant host (EAB eggs and larvae) for reproduction and establishment of the introduced parasitoids. After mortality of the larger ash trees, EAB densities collapse, and ash saplings, stump sprouts, and seedlings grow rapidly, supporting populations of EAB and the introduced parasitoids (Duan *et al.* 2014a; Kashian *et al.* in press). The establishment of *T. planipennisi* and *O. agrili* has also been confirmed after their release in 2011 at study sites in several aftermath forests of southeast Michigan where ash trees are regenerating and EAB densities are relatively low (L.S.B. and J.R.G., personal observation).

Parasitoid production at the United States Department of Agriculture Brighton EAB Biocontrol Facility has increased over the years due to improvements in rearing methods (Ulyshen *et al.* 2010a; Duan *et al.* 2011b, 2013c; Gould *et al.* 2011) and recent changes in release methods (Fig. 3). From 2007 to 2012, adult parasitoids were released onto the trunks of EAB-infested ash trees from small plastic cups provisioned with honey as their food source. By 2013, however, the parasitoid-release methods transitioned from the release of adults to the self-emergence of adults in the field, with *O. agrili* emerging from parasitised EAB eggs held in cups with screening, and *S. agrili* and *T. planipennisi* emerging from small ash bolts containing parasitised EAB larvae (United States Department of Agriculture 2013). The labour-intensive process of harvesting, feeding, and packaging the adult wasps provided more time for United States Department of Agriculture employees to focus their efforts on parasitoid production. Less demand for *S. agrili* (see section below on parasitoid establishment) also allowed for increased production of *O. agrili* and *T. planipennisi* (Fig. 3). As a consequence, hundreds of thousands of parasitoids have been

Fig. 3. Approximate number of parasitoids reared at the United States Department of Agriculture Emerald Ash Borer Biocontrol Facility in Brighton, Michigan since it became operational in 2009 through 2014. Data source: United States Department of Agriculture, Animal and Plant Health Inspection Service EAB Biocontrol Facility, Brighton (Michigan, United States of America).



available for release during the past few field seasons, with release sites in 19 American states; *T. planipennisi* were also provided to Canada for releases in Ontario starting in 2013 and in Québec the following year (Fig. 1).

Parasitoid establishment

A wide variety of sampling methods have been developed to evaluate EAB populations for parasitoid establishment and prevalence in the field one or more years after releases are completed (Duan *et al.* 2011a, 2012b, 2013b; United States Department of Agriculture 2013; Abell *et al.* 2014; Bauer *et al.* 2014). Sampling is done from late summer through early spring before parasitoid emergence begins (early April). If study objectives are to determine the presence or absence of the introduced parasitoids, adults of the three introduced species can be trapped in the field using yellow pan traps during late summer or reared from ash logs placed in rearing tubes in the laboratory following a period of chill to break obligate diapause in *O. agrili* and *S. agrili* (United States Department of Agriculture 2013). Sentinel eggs and larvae of EAB have been used for parasitoid recovery and to study their natural history, phenology, and abundance in the field (Duan *et al.* 2011a, 2012b). In addition, EAB eggs laid on trees by caged adults or laboratory-reared EAB eggs grafted onto trees have been used to establish cohorts for life table analyses (Duan *et al.* 2014a), assessment of woodpecker predation (Jennings *et al.* 2013), and the relation between tree diameter, bark thickness, and larval parasitism (Abell *et al.* 2012). However, to estimate the prevalence of EAB larval parasitism (*i.e.*, level of mortality caused to host population) in the field, ash trees must be felled and debarked, and EAB and parasitoids collected and returned to the laboratory. Immature parasitoids must be reared to the adult stage for identification, except for immature *Spathius*, which can be identified to species through genetic sequencing (Kuhn *et al.* 2013). Emerald ash borer larvae must be dissected under a microscope and examined for the presence of endoparasitoids (Duan *et al.* 2012a, 2012b, 2013a, 2014a). Estimating the prevalence of EAB egg parasitism in field populations is also labour intensive, but can be done successfully by collecting EAB eggs or bark samples from infested ash trees, and looking under the microscope at each EAB egg for the signs and symptoms of *O. agrili* (Abell *et al.* 2014).

At some of the first release sites in Michigan, the results of ongoing EAB-population dynamics studies confirm the sustained establishment of *T. planipennisi* and *O. agrili* from 2008 through 2014 (Duan *et al.* 2013a, 2014a; Abell *et al.* 2014; L.S.B. and J.J.D., personal observation). In Michigan, the three parasitoid species were released from 2007 to 2010 at six long-term study sites, each site being comprised of a release and control plot (at least 1 km apart). At these sites, EAB larval and egg parasitism have been determined annually since 2008. By fall 2012, Duan *et al.* (2013a) reported 92% of ash trees in release plots and 83% in controls had at least one EAB larva parasitised by *T. planipennisi*, with an average larval parasitism of 21% in release plots and 13% in control plots. From ash bark samples taken in 2012 and 2013, Abell *et al.* (2014) reported the percentage of sampled trees with *O. agrili*-parasitised EAB eggs was 28% in release plots and 11% in control plots, with an average EAB-egg parasitism of 19% in release plots and 9% in control plots. Overall, our research results demonstrate that populations of *T. planipennisi* and *O. agrili* are established, spreading, and increasing in prevalence in Michigan.

To assess establishment of the introduced parasitoids at non-research sites, EAB natural enemy surveys are performed by researchers or land managers at least one year after parasitoid releases are complete. As a result of these efforts, populations of *T. planipennisi* have been detected in other areas of Michigan including the Upper Peninsula (UP), and in Illinois, Indiana, Maryland, Minnesota, New York, Ohio, and Wisconsin (MapBioControl 2014; personal observation). In addition, populations of *O. agrili* have been found at other Michigan release sites and in the UP, and at other release sites in Indiana, Maryland, Minnesota, New York, Ohio, and Pennsylvania (MapBioControl 2014; personal observation). As in regions of China, *T. planipennisi* and *O. agrili* may play a critical role in the long-term management of EAB populations in surviving and regenerating ash.

Establishment of *S. agrili* in the United States of America, however, remains less certain. After its release at four Michigan EAB-infested sites in 2007, *S. agrili* was recovered in 18% of larvae at one of those sites the following spring, but none were found in 2009 (Gould *et al.* 2009). After seven years of sampling EAB populations at the six Michigan study sites, *S. agrili* parasitism was confirmed in only two EAB larvae at one site in 2011 (Duan *et al.* 2013a, 2014a; personal observation). However, *S. agrili* adults have been sporadically recovered in yellow pan traps at these and other release sites in Michigan (J.R.G. and L.S.B., unpublished data). A year or more after release, *S. agrili* was also recovered in Illinois, Indiana, Maryland, Ohio, Pennsylvania, New York, and Tennessee. Subsequent larval sampling in Maryland and Ohio revealed *S. agrili* has not persisted (L.S.B., J.J.D., and J.R.G., unpublished data). At this time, the status of possible sustained establishment of *S. agrili* remains unknown in the other states where it has been released.

The apparent lack of *S. agrili* persistence in many regions of the United States of America may result from an asynchrony between adult emergence and host availability (J.R.G., unpublished data) or limited cold tolerance for some northern regions (Hanson *et al.* 2013). Although *S. agrili* was found sporadically in northeast China (Liu *et al.* 2003), the population reared at the Brighton EAB Biocontrol Facility for release in the United States of America originated from Tianjin, China, a coastal city along the 39th parallel (J.R.G., unpublished data). This population of *S. agrili* may be better climate-matched to more southerly regions of the United States of America. In 2013 as a consequence of these findings, release of *S. agrili* was restricted to regions below the 40th parallel (United States Department of Agriculture 2013) (Fig. 1). Researchers and regulatory agencies in the United States of America are currently seeking approval for the introduction of a similar, large EAB larval parasitoid, *S. galinae* from the Russian Far East, which may be better climate matched and synchronised with EAB in northern regions of North America (Duan *et al.* 2012c; Federal Register 2015).

Conclusions

As EAB continues to invade areas of North America, high mortality of ash trees will continue due to limited EAB-host resistance and the lack of coevolved EAB natural enemies. Although researchers are seeking to understand EAB-resistance mechanisms in ash species, it may be decades before for EAB-resistant ash genotypes are available in North America. On the other hand, classical biological control of EAB was initiated within five years of the pest's discovery in Michigan in 2002, with the release of three EAB parasitoid species from China starting in 2007. The establishment and spread of two EAB biocontrol agents is now well documented at release sites in Michigan and other states, and researchers are continuing to monitor these parasitoid introductions as well as indigenous natural enemies on EAB population dynamics using life table analyses (Duan *et al.* 2014a). In addition, studies of ash survival, health, growth, and regeneration are also being performed at several long-term study sites in Michigan to assess the effects of EAB biocontrol in forested ecosystems (Kashian *et al.* in press). Similar studies are ongoing in New York following more recent parasitoid releases there (J.R.G., personal observation).

Researchers generally agree that the long-term management of EAB in North America will require an integrated approach that includes the use of classical biological control and silvicultural methods in forested ecosystems, systemic insecticides to protect high value trees in urban settings, and the development of EAB-resistant ash cultivars or hybrids (McCullough and Mercader 2012; Bauer *et al.* 2014; Herms and McCullough 2014). The high ash tree mortality rate created a strong selection event and may have favoured the selection of EAB-resistant ash genotypes. These surviving ash trees are being evaluated for innate resistance mechanisms. The introduced parasitoids may be crucial in supporting the continued survival of these ash trees, as well as ash seedlings, stump sprouts, and saplings developing in North American forests in the aftermath of the EAB invasion. Indeed, the longevity and condition of North American ash species planted in regions of China and the Russian Far East provide us with optimism. Continued foreign exploration for parasitoid species and genotypes in different regions of Asia, however, will be needed to provide effective natural enemies capable of establishing in the diverse climate zones of North America. Clearly, the ability of native, naturalised, and introduced parasitoids to suppress EAB population growth and ash mortality will require long-term evaluations over broad geographical regions as EAB continues to spread throughout North America

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