

Population dynamics of an invasive forest insect and associated natural enemies in the aftermath of invasion: implications for biological control

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Summary

1. Understanding the population dynamics of exotic pests and associated natural enemies is important in developing sound management strategies in invaded forest ecosystems. The emerald ash borer (EAB) *Agrilus planipennis* Fairmaire is an invasive phloem-feeding beetle that has killed tens of millions of ash *Fraxinus* trees in North America since first detected in 2002.

2. We evaluated populations of immature EAB life stages and associated natural enemies over a 7-year period (2008–2014) in six stands of eastern deciduous forest in southern Michigan, where *Tetrastichus planipennisi* Yang and two other Asian-origin EAB parasitoids were released for biological control between 2007 and 2010.

3. We observed $\approx 90\%$ decline in densities of live EAB larvae in infested ash trees at both parasitoid-release and control plots from 2009 to 2014 and found no significant differences in EAB density or mortality rates by parasitoids, avian predators or other undetermined factors between parasitoid-release and control plots. The decline in EAB larval density in our study sites was correlated with significant increases in EAB larval parasitism, first by native parasitoids, then by *T. planipennisi*.

4. Life table analyses further indicated that parasitism by the introduced biocontrol agent and the North American native parasitoids contributed significantly to the reduction of net EAB population growth rates in our study sites from 2010 to 2014.

5. *Synthesis and applications.* Our findings indicate that successful biocontrol of emerald ash borer (EAB) may involve suppression of EAB abundance both by local, generalist natural enemies (such as *Atanycolus* spp.) and by introduced specialist parasitoids (such as *T. planipennisi*). Biological control programmes against EAB in the aftermath of invasion should focus on establishing stable populations of *T. planipennisi* and other introduced specialist parasitoids for sustained suppression of low-density EAB populations. Moreover, we recommend releasing the introduced specialist biocontrol agents as soon as possible to prevent the outbreak of EAB populations in both newly infested and aftermath forests when EAB densities are still low.

Key-words: Hymenoptera, invasive, life table, natural enemies, parasitism, predation, wood borer

Introduction

Non-native species can achieve invasive pest status when they are accidentally moved to new locations if they become separated from their own natural enemy complexes and if local (indigenous) beneficial species (predators and/or

parasitoids) are unable to suppress them. Often, the most effective natural enemies of a non-native pest are those that co-evolved with it in its native range (van den Bosch, Messenger & Gutierrez 1982). Many dramatic successes in biological control have resulted from the introduction of natural enemies from the native ranges of invasive pests (Clausen 1978; Van Driesche *et al.* 2010).

The emerald ash borer (EAB) *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive forest pest

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that has killed tens of millions of ash (*Fraxinus*) trees in North America since its detection in 2002 in Michigan, USA, and Ontario, Canada (Herms & McCullough 2014). Shortly after its detection in North America, a classical biological control programme was initiated by the United States Department of Agriculture (USDA) against this invasive pest (Bauer *et al.* 2008). Subsequently, the U.S. regulatory authority (USDA Animal and Plant Health Inspection Service) approved environmental releases of three hymenopteran parasitoids of EAB that were collected from China, the pest's likely country of origin (Bauer *et al.* 2008, 2015; Bray *et al.* 2011). These exotic natural enemies co-evolved in Asia with EAB, including the solitary egg parasitoid *Oobius agrili* Zhang & Huang (Encyrtidae) and the two gregarious larval parasitoids *Tetrastichus planipennisi* Yang (Eulophidae) and *Spathius agrili* Yang (Braconidae). *Tetrastichus planipennisi* is an endoparasitoid that attacks late (3rd–4th) instars of feeding EAB larvae (Liu *et al.* 2007), while *S. agrili* is an ectoparasitoid of the same larval stages (Yang *et al.* 2005).

Several hundred thousand adult females (and proportional numbers of adult males) of these parasitoid species have been released at over 300 locations in 19 EAB-infested states and two Canadian provinces in North America (Mapbiocontrol 2014). While *S. agrili* has established in <5% of the sampled release sites, *O. agrili* and *T. planipennisi* are established with stable populations in >50% of release sites by autumn 2014 (Mapbiocontrol 2014). At the earliest release sites in North America (i.e. in Michigan), parasitism by both *O. agrili* and *T. planipennisi* increased from <1% in 2008, the first year after release, to 12–30% by 2012, c. 4 years after the last field releases (Duan *et al.* 2013a; Abell *et al.* 2014). To date, however, no assessment has been made to determine whether these introduced biological control agents have successfully suppressed EAB population growth in the 'aftermath' forests of southern Michigan.

During the same period (2008–2014), predation of immature EAB stages (larvae and pupae) by woodpeckers and other bark-foraging birds, and larval parasitism by native parasitoids (via new species associations) are frequently observed at sites in both the invasion's epicentre in Michigan (Lindell *et al.* 2008; Cappaert & McCullough 2009; Duan *et al.* 2010, 2014) and the expanding edge of the invasion (e.g. Illinois, Maryland, Pennsylvania, Ohio, New York) (Duan *et al.* 2013b; Jennings *et al.* 2013; Flower *et al.* 2014). In addition, putative host tree resistance, local pathogens and intraspecific larval competition cause some level of EAB larval mortality (Liu & Bauer 2006; Rebek, Herms & Smitley 2008; Duan *et al.* 2010, 2014; Tluczek, McCullough & Poland 2011; Jennings *et al.* 2013). The population control effect of these native biotic factors on EAB is currently unknown, as is the effect of establishment of the biological control agents. It is now timely to make such an assessment in regions such as Michigan where populations of the introduced larval parasitoid *T. planipennisi* are successfully established (Duan *et al.* 2013a).

Here, we report results of a 7-year study on population dynamics of immature EAB life stages and associated natural enemies in six deciduous forest stands in southern Michigan, where the three introduced biological control agents (*O. agrili*, *T. planipennisi* and *S. agrili*) were released from 2007 to 2010 as part of the biological control programme against EAB (Bauer *et al.* 2008; Duan *et al.* 2010, 2013a). Using separate estimates for EAB fecundity and egg mortality from laboratory data and other studies, we constructed life tables for EAB populations based on the observed counts of immature EAB stages and associated mortality agents, and estimated EAB net population growth rates for each forest site from 2008 to 2014 with and without the presence of parasitism by both native species and the introduced *T. planipennisi*. In addition, we quantified stage-specific mortality rates for factors affecting EAB larvae, including predation by avian predators, parasitism by both indigenous and introduced parasitoids, and an 'underdetermined' category that grouped putative host tree resistance, disease and intraspecific larval competition.

Materials and methods

STUDY SITES AND BIOLOGICAL CONTROL AGENTS RELEASED

Six forested sites from three southern Michigan counties were used in the current study (Duan *et al.* 2013a): Ingham Co. (three sites), Gratiot Co. (two sites) and Shiawassee Co. (one site). The sites in Ingham Co. consisted of two adjacent Meridian Township parks – Central and Nancy Moore Parks (site 1), Legg and Harris Nature Center Parks (site 2) and one county park – William M. Burchfield Park (site 3). The sites in Gratiot Co. were the Gratiot-Saginaw State Game Area (site 4) and the Maple River State Game Area (site 5), while the remaining site in Shiawassee Co. was Rose Lake Wildlife Area (site 6). Distances among these sites ranged from 10 to 60 km.

We divided each forest site into two plots (each >10 ha and separated by 1–6 km) and randomly designated them as either the biocontrol-release plot or the non-release control plot. Three introduced biocontrol agents – *O. agrili* (500–1330 gravid females), *S. agrili* (300–1220 females plus 150–500 males) and *T. planipennisi* (3300–3900 females plus 1000–1600 males) – were released at the centre of each release plot from 2007 to 2010. While the egg parasitoid *O. agrili* was released from June to August, the two larval parasitoids *S. agrili* and *T. planipennisi* were released from May to October. Detailed information on the release procedure, timing, frequency and number of adult wasps for each species at each study site can be found in Table S1 (Supporting information) (Duan *et al.* 2012a, 2013a; Abell *et al.* 2014).

SAMPLING PROCEDURES

Each year, from 2008 to 2014, we sampled populations of immature EAB life stages and scored mortality caused by parasitoids, avian predators and 'undetermined factors' at each study plot by destructively examining live ash trees that showed signs of EAB infestation (e.g. fresh woodpecker feeding, bark splits and epicormic shoots). Sampling occurred in late autumn (October–

November) with the exceptions of Ingham Co. sites 1–3 in 2008 (postponed until 25 April–12 May 2009) and Gratiot Co. site 5 in 2010 (postponed, due to flooding, until 20–25 April 2011). As the climate is cold from late autumn through early spring in Michigan, populations of both EAB and its associated parasitoids are relatively static during this period. Thus, the two delayed-sampling occasions were unlikely to result in biased estimates of EAB densities or associated parasitism. However, delayed sampling might have resulted in higher predation of overwintering EAB larvae and associated parasitoids by woodpeckers and other bark-foraging birds as these are active throughout the year (Duan *et al.* 2010; Jennings *et al.* 2013). Currently, there is no evidence that avian predators distinguish between unparasitized EAB larvae and those parasitized by various groups of hymenopteran parasitoids. Thus, the increased predation by avian predators because of the delayed sampling time from the autumn to early spring would have no effect on the marginal attack rate of EAB larvae by insect parasitoids (Elkinton *et al.* 1992; See Data Analysis).

At each sample date, 4–6 live ash trees [diameter at breast height (d.b.h.) = 7–21 cm] with symptoms of EAB infestation at each of the biological control release and control plots were felled and debarked using procedures described in Duan *et al.* (2012a, 2013a). Upon removing both outer and inner bark tissues from the main trunk and branches >3 cm in diameter, we examined each EAB gallery and pupation chamber (formed by mature J-shaped, 4th-instar larvae) and determined the stage and fate of each larva. EAB larval stages were characterized as small larvae (1st to 2nd instars with gallery width ≤ 2 mm) and large larvae (3rd to 4th instars including J-shaped mature larvae, gallery width >2 mm wide). The fate of each observed EAB larva was assigned to one of five categories: (i) development completed as evidenced by a D-shaped adult emergence hole, (ii) live immature stage, (iii) dead due to predation by avian predators (with bark and/or sapwood damage to galleries or pupation chambers by woodpeckers and other avian bark foragers), (iv) dead due to undetermined factors (e.g. host tree defenses, pathogens, intraspecific larval competition and weather) and (v) parasitized as indicated by the presence of eggs, larvae, pupae, cocoons or pharate adults of parasitoids in association with EAB larvae, cadavers or galleries.

Because parasitism is not always evident in the field, live EAB larvae were removed from the trees and dissected under a stereomicroscope in the laboratory to look for immature parasitoid stages or remains. Parasitoids were identified to species for the two endoparasitoids, *T. planipennisi* (gregarious) and *Phasgonophora sulcata* Westwood (Chalcididae) (solitary). Parasitism by the dominant group of native ectoparasitoids in the genus *Atanycolus* spp. (Braconidae) was identified with the presence of solitary parasitoid eggs, larvae or cocoons in EAB galleries. Other ectoparasitoids, including *Spathius* spp. (Braconidae), *Balcha indica* Mani and Kaul (Eupelmidae), *Eupelmus* spp. (Eupelmidae) and *Eurytoma* spp. (Eurytomidae), were found in association with EAB larval remains or cadavers in the galleries or pupation chambers only occasionally and pooled as ‘others’ for analysis.

DATA ANALYSIS

We used the mixed-effects linear model for analysis of variance (ANOVA) to evaluate differences in the resource-adjusted EAB density between parasitoid-release and non-release control treatments (SAS Institute 2014). The resource-adjusted EAB density was calculated as the mean count of individuals of all live EAB stages observed per unit (m^2) of the phloem area of sampled trees for

each plot in each sampling time (year). The total phloem area (y) of each sampled tree was estimated using a second-order polynomial model ($y = 0.024x^2 - 0.307x + 2.63$) as a function of the tree d.b.h. (x) (McCullough & Siegert 2007). The mixed-effects ANOVA model can be described as $Y_{ijk} = u + T_i + S_{j(i)} + P_k + (PT)_{ik} + e_{jk(i)}$, where Y_{ijk} = counts of individuals of all live EAB stages per m^2 of the phloem area in each sampling year; u = expected population mean over parasitoid-release and non-release control treatments; T_i = the fixed effect of sampling year; $S_{j(i)}$ = the random site effect within year; P_k = the fixed treatment (parasitoid-release vs. non-release control) effects; $(PT)_{ik}$ = fixed interaction effect between treatment (P_k) and sampling year (T_i); and $e_{jk(i)}$ = experimental residual effect. Similar mixed-effects linear ANOVA models were also used to evaluate differences in mortality rate of EAB larvae caused by different mortality factors (i.e. parasitoids, avian predators and undetermined factors). Mortality rates caused by different groups or species of larval parasitoids were calculated as marginal attack rates by excluding the number of EAB larvae preyed upon by avian predators and killed by undetermined factors. This calculation of marginal attack rate is based on the assumption that avian predators and agents in the ‘undetermined factor’ category acted on EAB larvae contemporaneously with the larval parasitoids and had no preference between healthy and parasitized EAB larvae (Elkinton *et al.* 1992). However, mortality rates by avian predators and undetermined factors were calculated as a proportion of the number of dead individuals from each cause relative to the total number of individuals (dead and live) from all EAB stages. Mortality rates from each observed factor were transformed with arcsine square root function before ANOVA; however, untransformed means are presented. JMP outputs along with statistical program scripts for all data analyses are presented in Appendix S1.

Life tables were constructed for EAB populations based on the observed numbers of immature EAB stages in each sampling year at each study site (pooled from both biological control release and control plots) by following the general methods and column definitions described in Southwood & Henderson (2000) and modified procedures in Duan *et al.* (2014). The procedures used to estimate the number of EAB individuals entering each stage and calculate net population growth rates are described in Duan *et al.* (2014). To quantify the impact the introduced biocontrol agent *T. planipennisi* and dominant indigenous parasitoids had on EAB population growth, we calculated the net EAB population growth rate (R_0) for life tables with and without parasitism from either the introduced biocontrol agent (*T. planipennisi*) or all the parasitoids (including introduced and North American native species or groups). We pooled data from both parasitoid-release and non-release control plots for each study site for the life table analysis because our data analyses failed to reveal any significant differences between parasitoid-release and non-release control plots in EAB densities and mortality rates caused by each of the three major factors (See Results).

Following approaches outlined in Duan *et al.* (2014), we calculated R_0 for EAB populations based on (i) the observed wild EAB immature stages and (ii) an estimated egg mortality rate of 30% to calculate an initial number of EAB eggs at the beginning of each generation for each study site. We attributed this level of EAB egg mortality mainly to the introduced egg parasitoid *O. agrili* as reported in Abell *et al.* (2014). To estimate the number of EAB eggs at the beginning of the next generation, based on the survivorship of observed individuals to adult stages, we used a sex ratio of 0.5 and an average of 30 viable eggs per

gravid female, as reported in Rutledge & Keena (2012). In addition, the complete predation rate of EAB larvae by avian predators was not fully measured by our autumn sampling scheme, as the same cohort of overwintering EAB larvae would suffer continued predation during winter. Duan *et al.* (2010) observed the number of late-instar EAB larvae preyed upon by woodpeckers and other bark foragers in both the autumn and the following spring. Based on those observations, we estimated that 30% additional mortality occurred in winter and we corrected our life tables by applying this rate of loss to late-instar larvae (except for sites sampled in the spring). However, we did not apply woodpecker predation (30%) to parasitized EAB larvae observed in the autumn. This is mainly because most parasitized EAB larvae were already in advanced stages (e.g. some had already become adults by the autumn) and these stages were no longer susceptible to woodpecker predation. We also included a 5% rate of mortality in the adult stage (mainly due to pathogens) (Duan *et al.* 2014). Collectively, these modifications allowed calculation of R_0 values. A mixed-effects ANOVA model, as described earlier, was used to detect the statistical significance of the effect of parasitism by both *T. planipennis* alone and in combination with the native parasitoids on EAB population growth rates.

Results

EAB DENSITIES IN BIOCONTROL-RELEASE AND CONTROL PLOTS

Patterns in the dynamics of live EAB densities (all larval instars including emerged adults) over the 7-year study period were similar between parasitoid-release and non-release control plots (Fig. 1). When adjusted to a per unit (m^2) of phloem area base (of sampled ash trees), the mean number of live EAB larvae of all instars increased from 16.1–20.4 in 2008 to 39.8–46.1 in 2009 and then declined $\approx 80\%$ to 8.9–7.8 in 2010 in both biocontrol-release and control plots. After this sharp decline, the EAB density resurged to 15.9–20.2 in 2011 and then trended downward from 7.0–8.9 in 2012 to 4.0–4.9 in 2014. ANOVA revealed

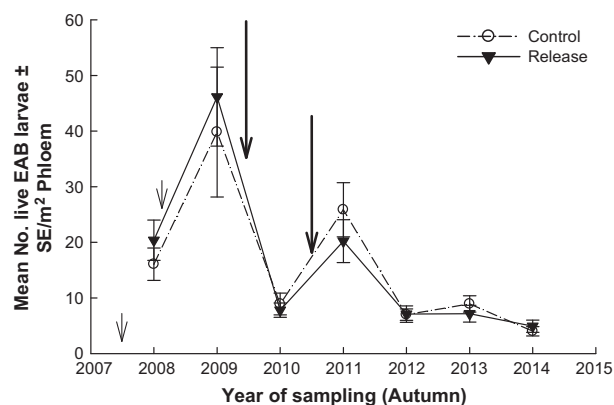


Fig. 1. Densities of live emerald ash borer (EAB) (all instars including emerged adults) per unit area (m^2) of sampled ash phloem in both biocontrol-release and control plots in Michigan during the 7-year study (2008–2014). Arrows indicate the timing of EAB parasitoid releases: small arrows represent low release numbers and large arrows high release numbers.

no significant differences in the mean EAB density between biocontrol-release and control plots ($P = 0.6244$), nor any significant interaction between biocontrol-release treatment and sampling year ($P = 0.1108$). However, EAB densities differed significantly among different sampling years ($P < 0.0001$).

MORTALITY OF EAB LARVAE CAUSED BY DIFFERENT GROUPS OF NATURAL ENEMIES

Three major parasitoid species or groups were consistently observed attacking EAB larvae in both biocontrol-release and non-release control plots at the study sites throughout the 7-year study period. These were principally the introduced biological control agent *T. planipennis* (Fig. 2a)

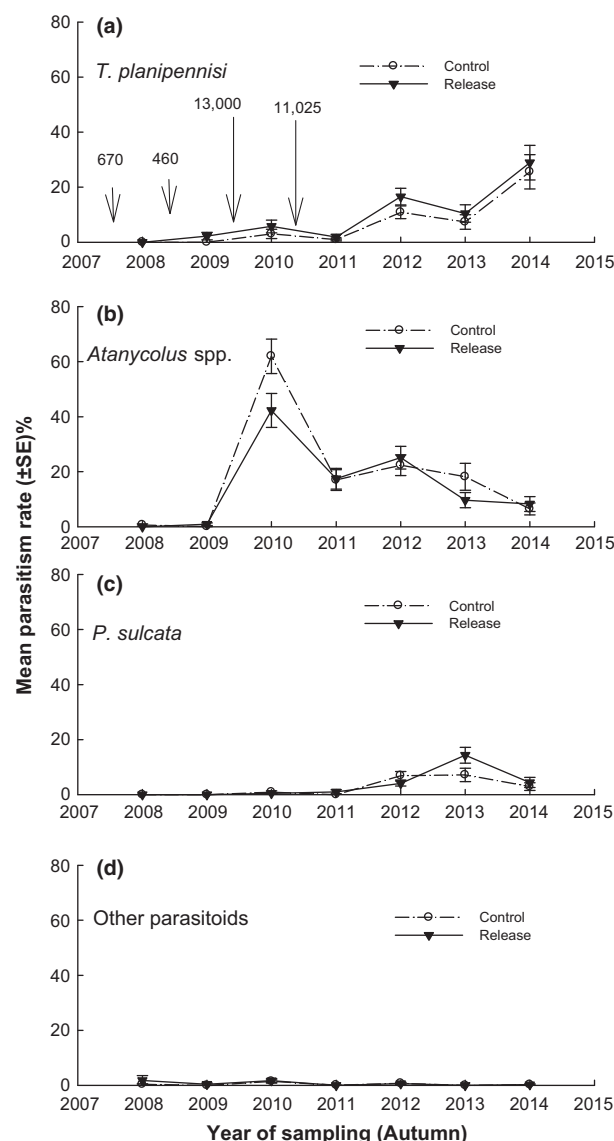


Fig. 2. Percentage parasitism of emerald ash borer (EAB) larvae by different groups of hymenopteran parasitoids observed in both the biocontrol-release and control plots in Michigan during the 7-year study (2008–2014). Each arrow represents the total number of *Tetrastichus planipennis* released annually.

and the North American native parasitoids *Atanycolus* spp. (Fig. 2b) and *P. sulcata* (Fig. 2c). The parasitism rate of EAB larvae by *T. planipennisi* was low (1.0–5.6%) between 2008 and 2011 in both release and non-release control plots, but increased to 10.9% and 16.6% in 2012 in the release and control plots, respectively. By autumn 2014, *T. planipennisi* parasitism rates were 25.6% and 28.9% in the release and control plots, respectively. In contrast, the parasitism rate of EAB larvae by *Atanycolus* spp. was <1% before 2008 and 2009 but increased sharply to 42–62% in 2010; thereafter, it showed continuous decline, dropping to 6.5–8.2% in 2014, in the release and control plots. Parasitism of EAB larvae by *P. sulcata* was also low from 2008 to 2011 (<1%), increased from 4.1–6.9% in 2012 to 7.2–14.4% in 2013 and then declined to 2.9–4.4% by 2014. In addition, low rates (<1%) of parasitism of EAB larvae by other groups of parasitoids such as *Spathius* spp. (including the introduced biocontrol agent *S. agrili*), *B. indica*, *Eurytoma* sp. and *Eupelmus* sp. were observed in both the release and control plots (Fig. 2d). ANOVA (see Appendix S1) revealed no significant differences in parasitism rates between the release and control plots by any of the species or groups (all $P > 0.10$), nor any significant interactions between biocontrol-release treatment and sampling year for any species or group of parasitoid (all $P > 0.10$). However, the parasitism rates by all groups of the observed parasitoids varied significantly among different sampling years (all $P < 0.0001$).

Unlike larval parasitism, EAB larval predation by birds was high (20–60%) throughout the study period in both biocontrol-release and control plots, but fluctuated with no apparent increase from 2008 to 2014 (Fig. 3a). Mortality of EAB larvae caused by undetermined factors (e.g. putative plant resistance, pathogens, intraspecific larval competition, weather) also fluctuated throughout the study period with increases from 6–14% in 2008 to 19–28% in 2014 (Fig. 4b) in both biocontrol-release and control plots. ANOVA (see Appendix S1) again revealed no significant differences in both rates of avian predation and mortality rates caused by undetermined factors between the release and control plots (all $P > 0.10$), nor were there any significant interactions between biocontrol-release treatment and sampling year in either rates of avian predation or mortality from undetermined factors (all $P > 0.10$). Again, the mortality rates caused by both avian predators and undetermined factors varied significantly among different sampling years (all $P < 0.0001$).

IMPACT OF INTRODUCED BIOCONTROL AGENT ON EAB NET POPULATION GROWTH RATE (R_0)

A representative life table of the EAB population based on the observed immature stages from one of the six study sites (combined release and control plot data) in the autumn of 2014 is presented in Table S2. This life table contains details of apparent (stage-specific) mortality and

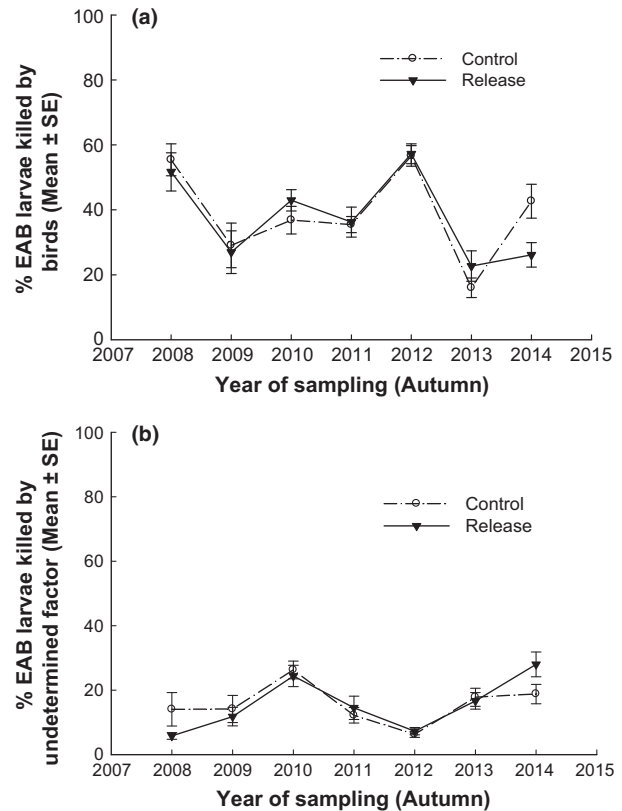


Fig. 3. Predation rates of emerald ash borer (EAB) larvae by birds (a) and mortality rates caused to EAB larvae by undetermined biotic factors such as putative host tree resistance, disease and/or intraspecific competition in foragers (b) in both biocontrol-release and control plots in Michigan during the 7-year study (2008–2014).

associated mortality factors, real mortality and estimates of net population growth rate (R_0) for EAB populations in the autumn of 2014 at Rose Lake State Wildlife Area (site 6). Impacts of a specific mortality factor on EAB population growth can be assessed by changes in R_0 values when that mortality factor is removed from the life table under the assumption that all subsequent mortality factors kill the same percentage of EAB (i.e. none are density dependent). For example, when parasitism by the introduced biocontrol agent *T. planipennisi* was removed from this life table, R_0 values increased *c.* 74% from 1.23 to 2.11, indicating that parasitism by *T. planipennisi* contributed a 42% reduction in EAB population growth at this site.

Data from life tables constructed for the complete generation observed each year at the six study sites (pooled from biocontrol-release and control plots at each site) showed that the average R_0 values decreased nearly 76% from 3.53 (the peak) in 2009 to 0.86 in 2010 (Fig. 4). After 2010, R_0 values resurged to 2.56 in 2011 and then fluctuated downward again, being $R_0 = 1.28$ in 2012 and $R_0 = 1.73$ in 2014. When parasitism by *T. planipennisi* was removed from the life table analysis, changes to R_0 values were negligible until 2012 (Fig. 4), indicating a

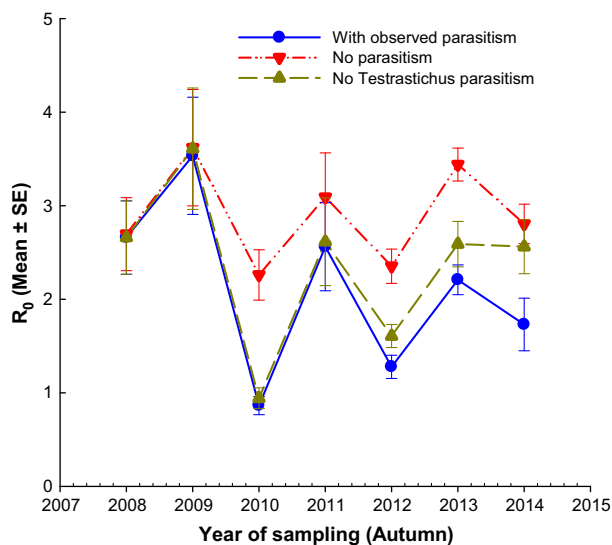


Fig. 4. Net population growth rates (R_0) of emerald ash borer (EAB) populations estimated from life tables of each observed generation across different study sites in Michigan during the 7-year study (2008–2014).

minimal effect by this agent on EAB population growth during the first 3 years after its release (2007–2010). However, EAB population R_0 values, when *T. planipennisi* parasitism was removed from life tables, increased *c.* 25% between 2012 and 2013, and 50% by 2014, indicating an increased impact of this biological control agent on EAB population growth over time. Further life table analyses also showed that the major native EAB larval parasitoids (*Atanycolus* spp. and *P. sulcata*) played a large role in reducing R_0 values, prior to 2014 (Fig. 4 – dotted line). ANOVA (Appendix S1) revealed significant effects of parasitism by *T. planipennisi* and in combination with the native parasitoids on EAB population growth rates, as well as significant interaction between parasitism by these natural enemies and sampling years.

Discussion

Seven years of field data demonstrate that the nearly 90% decline in EAB density from 2009 to 2014 was correlated with a significant increase in larval parasitism, first by the native parasitoids *Atanycolus* spp. and *P. sulcata* and then by the introduced biocontrol agent *T. planipennisi*. Our life table analyses further demonstrated that both native natural enemies and the introduced biocontrol agent *T. planipennisi* played significant roles in slowing the population growth of EAB. These findings strongly suggest that successful biological control of EAB in the outbreak phase of its invasion may be dominated by local, generalist natural enemies (such as woodpeckers and native hymenopteran parasitoids) but that this may gradually shifted toward increasing prevalence of introduced specialist parasitoids such as *T. planipennisi* as its impact on net EAB population growth rate increased. These specialized species will most

likely be the natural enemies that remain important in the more stable, lower density aftermath populations of EAB, but this has yet to be determined. In such aftermath EAB populations, such as those now present in southern Michigan, biological control programmes should focus on establishing widespread populations of *T. planipennisi* and other introduced specialist parasitoids including *O. agrili* and *S. agrili* or *S. galinae* throughout the EAB-infested region. In newly infested areas where EAB populations are still low, introduced specialist biocontrol agents should be released as soon as possible to slow EAB population growth rate and rapid, widespread ash tree mortality. Similar processes were at play in the dramatically successful classical biocontrol effort against winter moth *Operophtera brumata* (L.), in which this forest pest was suppressed by the interaction between generalist predators of pupae in the soil and introduced specialist parasitoids (Roland 1994; Roland & Embree 1995).

Results from our study showed that mortality rates of immature EAB stages caused by avian predators (20–60%) and undetermined factors (6–28%) were high to moderate throughout the study period, but fluctuated with no apparent increase and were not correlated with the decline in EAB density. In contrast, parasitism by the newly released biological control agent *T. planipennisi* and the North American native parasitoids *Atanycolus* spp. and *P. sulcata* increased sharply after EAB densities peaked in 2009. Increases in total EAB larval parasitism by these parasitoids after 2009 were correlated with the subsequent decline in EAB density in both the biocontrol-release and control plots. However, the dynamics of parasitism by the indigenous species or groups of parasitoids and the introduced biocontrol agent continued to change after 2010. Whereas parasitism by *T. planipennisi* continued to increase as EAB densities declined, parasitism by generalist parasitoids *Atanycolus* spp. and *P. sulcata* declined by 2014.

The decline in EAB larval parasitism by these North American native parasitoids (*Atanycolus* spp. and *P. sulcata*) may be related to the decline in the density of live EAB larvae observed in our study sites and their decreasing success in host location rather than interspecific competition with the introduced biocontrol agent *T. planipennisi*. It is known that *Atanycolus* spp. and *P. sulcata* are generalists that attack many groups of wood-boring beetles before discovering EAB as a host in North America (Marsh, Strazanac & Laurusonis 2009; Taylor *et al.* 2012). In contrast, *T. planipennisi* is a specialist co-evolved with EAB in north-east Asia, and its known host range only includes EAB (Bauer *et al.* 2015). It is generally agreed that generalist predators and parasitoids are often opportunistic and exploit hosts occurring at high densities, while specialist predators or parasitoids have high fidelity to their co-evolved hosts and are more efficient in exploiting low densities of their target hosts or prey (see review in Symondson, Sunderland & Greenstone 2002). In addition, we observed only a few (3) incidences

of co-parasitism by both *T. planipennisi* and *Atanycolus* spp. or by *T. planipennisi* and *P. sulcata* throughout 7 years of the study. Recent laboratory studies also demonstrate that adult *T. planipennisi* strongly discriminate against EAB larvae parasitized by other species even in small laboratory cages (Yang *et al.* 2013).

The lack of significant differences in EAB densities and mortality rates caused by the natural enemies between the biocontrol-release and non-release control plots over the 7-year period reflect a common challenge faced by many biological control programmes for evaluation of impacts of introduced biocontrol agents on targeted pest populations. In our study, the non-significant differences in both the pest density and parasitism rate were clearly due to the lack of measurable impacts from the introduced agent *T. planipennisi* in the earlier phase of the biocontrol programme (from 2008 to 2011) and then to the spillover effect of this agent dispersing into the control plots which were relatively close by; this was particularly evident in the later years of our study (from 2012 to 2014). Although increased distances between biocontrol-release and control forest plots could potentially help reduce or delay the spillover effects of dispersing biocontrol agents, large variations in habitat characteristic such as ash tree composition and climatic conditions between distant forest plots could also confound the biological control treatment effect. As demonstrated in the current study as well as previous ones (e.g. Van Driesche & Taub 1983; Jennings *et al.* 2013; Duan *et al.* 2014), construction of life tables of the targeted pest population and subsequent analyses of net population growth rates with and without removal of the biological control agent's effect from the life table provides a feasible and powerful tool for quantifying the contribution of biological control agents or other factors on the dynamics of target pest populations.

Once it has spread to an ash-dominant forest, EAB generally requires a few years before its density starts to rise rapidly, and thereafter, it rapidly kills most of the large (mature) ash trees in the stand (Burr & McCullough 2014; Herms & McCullough 2014). With the depletion of host tree resources due to the high level of ash tree mortality, the absolute EAB density at a site (e.g. per hectare) would decline sharply, resulting in a shrinking population (i.e. $R_0 < 1$, see reviews in Herms & McCullough 2014). The decline in the resource-adjusted EAB density, as reported in our study, must be attributed in part to this general collapse of EAB populations follow widespread ash death. Depletion of host tree resources in a local area, such as our study sites, would cause EAB adults at some point to disperse in search of more abundant hosts (e.g. Mercader *et al.* 2009; Siegert *et al.* 2010). However, many small ash trees and saplings (d.b.h. range 1.0–15 cm) are still abundant and susceptible to EAB infestation in our study sites. From the point of view of these surviving ash trees, the pest pressure they now experience is greatly reduced, increasing the prospect for their continued survival. In our view, the reduction of ash resources alone in

our study sites did not fully explain the nearly 90% decrease in the resource-adjusted viable EAB densities per m² of ash phloem from 2009 to 2014.

Although the R_0 values of EAB populations in our study sites were still greater than one in 2014 ($R_0 = 1.73$), EAB densities at the current level have not killed many young ash trees and/or saplings in southern Michigan (Kashian & Witter 2011; L.S. Bauer, unpublished data). As the introduced larval parasitoid *T. planipennisi*, as well as the egg parasitoid *O. agrili* (not analysed in this study, but see Abell *et al.* 2014), continues to establish stable populations and their impact on EAB population dynamics continues to increase, it is possible that EAB in the aftermath of its invasion may be prevented from reaching tree-killing densities again in the aftermath forests of southern Michigan. However, we caution that this dynamic is likely to change as the current young ash trees and saplings at our study sites grow to larger size classes (d.b.h. > 15 cm). Previous study showed that the efficiency of *T. planipennisi* in attacking EAB larvae feeding in the main trunks is drastically reduced in ash trees with d.b.h. > 12 cm because of this species' short ovipositor (1.2–2.5 mm) (Abell *et al.* 2012). Therefore, we suggest that additional guilds of natural enemies, such as *S. galinae* with a considerably longer ovipositor (4–5 mm) as reported in Duan, Yurchenko & Fuester (2012b), be introduced to North America to complement the role of *T. planipennisi* in regulating EAB populations associated with large-diameter ash trees.

Finally, we acknowledge that other mortality factors, such as extreme weather events, also may affect the population growth and range expansion of EAB (Liang & Fei 2014). During our study, unusually cold conditions were experienced during the winter of 2011 but with no obvious effects on over-wintering EAB larvae or associated parasitoids. We speculate that such weather-related mortality would more likely affect the exposed stages (adults and eggs) but have lesser effect on the larval and pupal stages under the bark that are buffered from extreme weather conditions (Vermunt *et al.* 2012). Further studies are needed to determine the degree to which physical conditions affect the dynamics of EAB and associated parasitoid populations.

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Data accessibility

Data are available through the Ag Data Commons (National Agricultural Library, USDA Agricultural Research Service): <http://dx.doi.org/10.15482/USDA.ADC/1178758> (Duan *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Release time, frequency and number of introduced emerald ash borer parasitoids released at study sites.

Table S2. A representative life tables for emerald ash borer populations.

Appendix S1. JMP scripts and output on ANOVA (mixed-effects model) for emerald ash borer larval densities, parasitism rates by different species or groups of native and introduced parasitoids, bird predation rates, mortality rate caused by undetermined factor, and emerald ash borer net population growth rates.