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Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States

Jian J. Duan · Leah S. Bauer ·
Kristopher J. Abell · Roy van Driesche

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Abstract Populations of hymenopteran parasitoids associated with larval stages of the invasive emerald ash borer (EAB) *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) were surveyed in 2009 and 2010 in the recently invaded areas in north central United States (Michigan), where two introduced EAB larval parasitoids, *Tetrastichus planipennisi* Yang and *Spathius agrili* Yang were released for classical biological control. Results from two years of field surveys showed that several hymenopteran parasitoids have become associated with EAB in Michigan. Among these parasitoids, the gregarious species *T. planipennisi* was the most abundant, accounting for 93% of all parasitoid individuals collected in 2009 (immediately after field release) and 58% in 2010 (a year later after field releases). Low levels (1–5%) of parasitism of EAB larvae by *T. planipennisi* were consistently detected at survey sites in both years. Separately, the abundance of the native parasitoid, *Atanycolus* spp., increased sharply, resulting in an average parasitism rate of EAB larvae from <0.5% in 2009 to 19% in 2010. Other parasitoids such as *Phasgonophora sulcata* Westwood, *Spathius* spp., *Balcha indica* Mani & Kaul, *Eupelmus* sp., and *Eurytomus* sp. were much less abundant than *T. planipennisi* and *Atanycolus* spp., and each caused <1% parasitism. Besides hymenopteran parasitoids, woodpeckers consumed 32–42% of the immature EAB stages present at our study sites, while undetermined biotic factors (such as microbial disease and host tree resistance) caused 10–22% mortality of observed EAB larvae. Relevance of these findings to the potential for biological control of EAB in the invaded areas of North America is discussed.

Keywords Indigenous · Exotics and invasive species · Parasitoids · Wood borers · Biological control

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J. J. Duan (✉)
USDA ARS, Beneficial Insects Introduction Research
Unit, Newark, DE 19713, USA
e-mail: jian.duan@ars.usda.gov

L. S. Bauer
USDA Forest Service, Northern Research Station,
East Lansing, MI 48823, USA

K. J. Abell
Department of Plant, Insect and Soil Sciences,
University of Massachusetts, Amherst, MA, USA

R. van Driesche
Department of Plant, Insect and Soil Sciences,
University of Massachusetts, Amherst, MA, USA

Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), native to northeast Asia, was first discovered in southeast Michigan (USA) in 2002 (Haack et al. 2002), and has since spread to 15 U.S. states (Emerald Ash Borer Information 2011) and two Canadian provinces (Canadian Food Inspection Agency 2011). Much has been learned in the past several years about the biology and life history of this pest and various biotic factors that could potentially affect its population dynamics in North America. In most of the infested areas, EAB adults emerge in late spring and early summer (May–June), and feed on ash foliage for at least two weeks before mating and laying eggs in crevices and under bark flakes on limbs and trunks of ash trees in early summer (June–July). After eclosion, first instar larvae chew through the bark to reach the phloem, where they feed and develop throughout the growing season (Cappaert et al. 2005). After larvae go through three molts, the mature fourth instar larvae chew pupation chambers in the outer sapwood or bark. The mature larvae then develop a folded appearance, which we termed J-larvae. Usually following a winter chill period, J-larvae develop into prepupae, which are visibly shorter and more cylindrical. Pupation generally occurs in early spring, with adults emerging from late spring through early summer. Adults live for several weeks feeding on mature ash leaves, and rarely cause any significant damage to the host tree (Bauer et al. 2004). Larvae, in contrast, feed for several months on ash tree phloem, creating extensive galleries under the bark. When EAB populations are high, larval consumption of tree phloem is substantial, resulting in tree girdling and death in three to five years.

EAB populations in the northern United States (e.g., Michigan and Pennsylvania) may require two years to complete development, especially in newly infested, healthy ash trees (Cappaert et al. 2005; Siegert et al. 2007; Duan et al. 2010). Similar developmental plasticity is known for *Agrilus anxius* Gory, a native buprestid that feeds on birch trees (Barter 1957). The most recent observations in Michigan indicate that EAB populations have overlapping two-year generations in the field, with a mixed population of first-season (1st to 3rd instar larvae) and second-season (4th instar larvae to prepupae or pupae) immature stages being present in both spring and fall (Duan et al. 2010). One implication of this condition is that larval stages suitable for parasitism are likely to be present for nearly the entire growing season, favoring the impact of biological control agents. Field surveys in Michigan, Pennsylvania, Ohio, USA and Ontario, Canada show that EAB larvae in newly infested areas are parasitized by several native North American species or self-introduced exotic species of Hymenoptera, including *Atanycolus* spp., *Spathius floridanus* Ashmead (=*S. simillimus* Ashmead), *Spathius laflammei* Provancher, and *Leluthia astigma* (Ashmead) (Braconidae), *Phasgonophora sulcata* Westwood (Chalcididae), *Balcha indica* Mani & Kaul and *Eupelmus* spp. (Eupelmidae) (Bauer et al. 2004, 2005; Lyons 2008; Cappaert and McCullough 2009; Duan et al. 2009; Kula et al. 2010). The highest known level of mortality, however, is caused by woodpeckers, which attack the larger, immature stages of EAB in North America (Cappaert et al. 2005; Lindell et al. 2008; Duan et al. 2010). Host-tree resistance and pathogenic microorganisms also influence the survival and reproduction of EAB in both North America (Bauer et al. 2004; Rebek et al. 2008; Duan et al. 2010) and Asia (Liu and Bauer 2006; Liu et al. 2007; Duan et al. 2011). Although the combined rate of attack by these various mortality agents is inadequate to suppress EAB spread and ash mortality in North America, their potential role in regulating population dynamics may increase over time and needs to be investigated for the purpose of managing this invasive pest.

Besides these endemic biotic factors, the recent classical biological control efforts against EAB in North America have also led to the release of one egg parasitoid, *Oobius agrili* Zhang and Huang (Encyrtidae), and two larval parasitoids, *Tetrastichus planipennisi* Yang (Eulophidae) and *Spathius agrili* Yang (Braconidae), all of which were collected from northern China (Liu et al. 2003; Yang et al. 2005, 2006; Zhang et al. 2005; Liu et al. 2007). Releases of the Chinese parasitoids started in Michigan in 2007, followed by Ohio and Indiana in 2008, and Maryland and Illinois in 2009 and 2010 (USDA APHIS 2007; Bauer et al. 2008, 2010). Although these parasitoids have apparently become established at several locations, levels of parasitism by these introduced parasitoids at the release sites are considerably lower ($\approx$1.5% in Duan et al. 2010) than levels (12–73%) reported from China (Liu et al. 2003, 2007). However, it is expected that prevalence of these introduced biological control agents and other factors will change over time. Successional changes in the endemic parasitoid complex attacking EAB larvae in the United States and their interactions with the newly introduced EAB larval parasitoids from China can now be studied in the field.

For the last two years, we conducted field surveys to characterize the hymenopteran parasitoids associated with EAB larvae infesting North American ash trees (primarily *Fraxinus pennsylvanica* Marshall) in recently invaded areas of Michigan. The surveys were conducted in 2009 and 2010 in three forested areas of central Michigan, where the two newly introduced larval parasitoids, *T. planipennisi* and *S. agrili,* were released in 2008 and 2009.

Materials and methods

Survey sites

Surveys were conducted in ash stands in naturally forested areas located at three sites in Ingham County, near Lansing, MI, USA. The characteristics of the three sites were described in detail in Duan et al. (2010). Site 1 (42° 43′N/84° 25′W) was located in two contiguous Meridian Township parks (Central Park and Nancy Moore Park). Site 2 (42° 41′N/84° 22′W), *ca*. 8-km away, spanned two additional Meridian Township parks (Harris Nature Center and Legg Park). Site 3 (42° 34′N/84° 36′W) was located in the William M. Burchfield County Park in Holt, MI, USA, *ca*. 32 km southwest of the other two study sites. At each site, two four-hectare plots, separated from each other by *ca*. 1 km, were selected and randomly assigned as either: (1) a parasitoid release treatment plot or (2) a non-release control plot. These sites were primarily early successional, second-growth northern deciduous forest dominated by green ash (*F. pennsylvanica*). Less abundant trees species in these forests were: white ash (*F. americana* L.), black ash (*F. nigra* Marsh), red maple (*Acer rubrum* L.), boxelder (*A. negundo* L.), oak (*Quercus* spp.), black cherry (*Prunus serotina* Ehrh.), poplar (*Populus* sp.), black walnut (*Juglans nigra* L.), cottonwood (*Populus deltoides* Bartr. ex Marsh), basswood (*Tilia america* L.), and some conifers, such as spruce (*Picea* spp.) and pine (*Pinus* spp.).

Parasitoid releases

During the summer (13 August to 4 October) of 2008, 80–100 females (plus 50–100 males) of each of the two introduced emerald ash borer larval parasitoids, *T. planipennisi* and *S. agrili,* were first released in each of the three release plots at each site. Further releases (made frequently in small batches) of approximately 3200 females (plus 1000–2000 males) of *T. planipennisi* and a single release of 200 females (plus 100 males) of *S. agrili* were also made during the summer 2009 in the release plot at each study site. Both sexes of *T. planipennisi* or *S. agrili* were held together inside rearing cages for at least three days before field releases, and presumably mated as both species normally mate immediately upon adult wasp emergence (Ulyshen et al. 2010; Gould et al. 2011). Detailed procedures, timing, and number of the parasitoids released for each species at the three study sites are described in Duan et al. (2010).

Sampling methods

Surveys of parasitoid populations associated with EAB larvae started in the spring (25 April–12 May) of 2009 and continued into the summer (20–24 July) and fall (29 September–7 October) of 2009. In 2010, surveys continued in the spring (20–25 April), summer (July 21–25), and fall (October 19–22). At each survey time, we destructively sampled three to five ash trees with apparent symptoms of EAB infestations (e.g., bark splits, presence of woodpecker scaling and feeding damage, and epicormic growth on the main trunk) from each parasitoid release plot and non-release control plot, respectively. These sampled trees sustained moderate to heavy injuries by EAB larvae. For sampling, ash trees were felled using a bow saw, and all sections of the tree including the main trunk and all branches >3 cm in diameter were debarked with a draw knife and examined for presence of immature stages of EAB and associated parasitoids. To avoid damaging immature EAB larvae, prepupae and/or pupae 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. The exposed immature EAB stages and associated parasitoids were collected with featherweight forceps (Bioquip Products #4750), 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 parasitoids were returned either to the USDA ARS Beneficial Insects Introduction Research Unit quarantine facility (BIIR, Newark, DE, USA) or the USDA Forest

Service Northern Research Station laboratory (East Lansing, MI, USA) to rear out parasitoid adults or dissect out larval parasitoids. The ectoparasitoids collected by debarking trees were immature stages, including larvae and/or pupae with or without parasitoid cocoons, stages which were readily visible to observers under field conditions. For the endoparasitoids *P. sulcata* or *T. planipennisi*, EAB larval parasitism was determined by either rearing or dissection using a dissecting microscope. EAB stages damaged during the sampling process were collected into culture plates in the field, refrigerated, and dissected within one or two days for presence of eggs or larvae of the internal parasitoids *P. sulcata* and *T. planipennisi*. Adult parasitoids recovered from rearing in the laboratory were sent to the USDA ARS Systematic Entomology Laboratory (SEL) or Paul Marsh, Inc. (North Newton, KS, USA) for identification. Voucher specimens were returned and deposited at the USDA ARS BIIR (Newark, DE, USA) or the Michigan State University Arthropod Research Collection (East Lansing, MI, USA).

In addition to recording parasitism, we recorded two other categories of mortality associated with EAB immature stages: (1) woodpecker predation and (2) undetermined biotic factors (e.g., disease, host plant resistance). Woodpecker predation was determined by examination of the bark and outer sapwood for the characteristic woodpecker holes caused by woodpecker feeding and missing or, in some cases, partially consumed EAB larvae in close proximity to these holes. These characteristics allowed us to quantify woodpecker predation (Lindell et al. 2008). Deaths of cadavers that were associated with mold (pathogenic and/or saprophytic) were rotten and foul smelling or were covered with plant callus tissue. They were grouped as "undetermined biotic factors" and could not be separated further (Duan et al. 2010). We also observed some lethargic, limp, and motionless larvae lacking the above symptoms and those larvae were returned to the laboratory and either placed on artificial diet or dissected under dissecting microscope to check for endoparasitoids.

Data analysis

To estimate the density of EAB, we calculated the phloem area of each sampled tree using the method described by McCullough and Siegert (2007). Briefly, this method requires measuring the diameter at breast height (DBH) of each tree (x) in the field, and using the second-order polynomial function to estimate the phloem area (y): $y = 0.24x^2 - 0.307x + 2.63$. The density of immature EAB (larvae, J-larvae, prepupae, and pupae) was calculated as the number of immature EAB per m^2 of sampled phloem. EAB densities were then square-root transformed for two-way analysis of variance (ANOVA) with parasitoid treatment (release vs. non-release) and study site as two main factors (effects) for both 2009 and 2010. Least square mean difference (LSD) Student's *t*-tests were used to separate differences in EAB densities among different study sites for each year. Percent mortality caused by woodpecker predation and undetermined biotic factors was calculated as a proportion of the total number of immature EAB life stages collected from each ash tree. These values were then transformed with an arcsine square root function for two-way analysis of variance (ANOVA) with parasitoid release treatment (release vs. non-release) and study site (Burchfield, Central/Nancy Moore, and Harris/Legg) as the two main factors (effects). We used a split-plot design to test for differences in EAB mortality among different study sites and between the parasitoid release and non-release (control) plots for each year.

To calculate the percentage of EAB parasitism for each parasitoid species, we excluded those immature EAB stages that were attacked by woodpeckers from the denominator (total sample sizes) because we could not able to determine if those missing EAB had been healthy or parasitized before their removal. Percentage parasitism values for different parasitoids were arcsine-square root transformed and then analyzed with a three-way ANOVA model for comparisons among different sampling seasons, parasitoid release treatment (release vs. non-release), and study sites. Statistical analyses were performed using JMP 8.0.1 (SAS Institute Inc 2008).

Results

EAB density and mortality caused by woodpeckers and undetermined biotic factors

A total of 6,439 and 6,566 immature EAB were observed from 54 and 40 green ash trees (mean DBH ranged from 8.1 to 11.7 cm) in 2009 and 2010, respectively. Across different study sites, mean EAB density (number of individuals m^{-2} phloem) ranged from 87.1 to 126.5 in 2009 and 63.9 to 90.2 in 2010 (Table 1). From 32 to 42% of immature EAB stages were removed from feeding galleries and/or pupal chambers by woodpeckers (Table 1). Additionally, another 10–22% of EAB larvae in various instars died of undetermined biotic factors. There were no significant differences in EAB densities, woodpecker predation, or mortality rate from undetermined biotic factors among study sites in either 2009 or 2010 (LSD student's tests $P > 0.05$).

EAB larval parasitoids

Eight different groups of hymenopteran parasitoids were observed parasitizing EAB larvae in both 2009 and 2010 at the study sites (Table 2). These were *T. planipennisi*, *Antanycolus* spp., *P. sulcata*, *Spathius* spp., *B. indica, Eupelmus* sp., *Eurytomus* sp., and *Dolichomitus* sp. The newly introduced gregarious biological control agent *T. planipennisi* was the most abundant species based on the number of individuals collected, accounting for 93.8% and 58.1% of all parasitoid individuals collected in 2009 and 2010, respectively. However, average (annual) parasitism rate of EAB larvae by *T. planipennisi* (across all sampling seasons) was still low, ranging from approximately 0.8% in 2009 to 1.2% in 2010. The high relative abundance and low parasitism rate of *T. planipennisi* was largely due to the large brood size of this gregarious species. In both survey years, parasitized EAB larvae produced an average (±SE) of 30 (±6.0) *T. planipennisi* progeny per host ($n = 20$ for 2009; $n = 49$ for 2010), with a female-to-male sex ratio of approximately 3:1.

In contrast, the relative abundance (proportion) of the solitary ectoparasitoids in the group *Antanycolus* spp. increased from 1.3% in 2009 to 36.7% in 2010 (Table 1). The annual average parasitism of EAB larvae by *Atanycolus* spp. across all study sites increased from approximately 0.6% in 2009 to 19.4% in 2010 (Table 1). Based on taxonomic identification of the emerging adults ($n = 383$), *A. cappaerti* Marsh and Strazanac accounted for nearly 93% of the *Atanycolus* adults reared. Other species recovered were *A. hicoriae* Shenefelt (5%), *A. tranquebaricae* Shenefelt (1%) and *A. disputabilis* Cresson (<1%). Unlike *T. planipennisi*, the sex ratio of *A. cappaerti* was male-biased 0.6:1 (female:male).

The solitary endoparasitoid *P. sulcata* accounted for 3.4% of all the parasitoid individuals collected in 2009, and 1.3% in 2010. In both years, however, the combined annual average parasitism of EAB larvae by *P. sulcata* was <1%. The gregarious ectoparasitoid group *Spathius* spp. accounted for 0.9 and 2.4% of all parasitoid individuals reared in 2009 and 2010, respectively, with an average parasitism <0.3% for both years. All adult wasps ($n = 37$) that emerged from the *Spathius* spp. cocoons ($n = 50$) collected from our study sites were identified as *S. floridanus*, a native braconid, not the introduced *S. agrili*. Only one individual of the non-native solitary ectoparasitoid *B. indica* was collected in 2009 from one of the study

Table 1 Density and mortality of emerald ash borer (EAB), *Agrilus planipennis*, larvae caused by woodpeckers and undetermined biotic factors (such as disease, starvation, competition, and/or host plant resistance) in study sites near Lansing, Michigan, USA

Year	Study sites	Number of trees sampled	Mean ± SE DBH (cm) of sampled trees[a]	Mean ± SE EAB larvae (n) m^{-2} phloem[a]	% ± SE EAB larvae (n) preyed on by woodpeckers[a]	% ± SE EAB larvae (n) killed by undetermined biotic factors[a]
2009	Burchfield	18	9.1 ± 0.7b	126.5 ± 22.9a	39.3 ± 6.3a	11.1 ± 4.6a
	Central/Nancy Moore	19	11.5 ± 1.2a	87.1 ± 13.7a	34.4 ± 5.1a	19.8 ± 5.2a
	Harris/Legg	17	11.7 ± 1.0a	99.9 ± 23.2a	42.2 ± 6.5a	9.9 ± 2.7a
2010	Burchfield	12	8.1 ± 0.5b	90.2 ± 13.9a	40.8 ± 5.5a	13.3 ± 2.9a
	Central/Nancy Moore	14	9.9 ± 0.8ab	63.9 ± 14.9a	39.4 ± 5.3a	22.0 ± 3.9a
	Harris/Legg	14	11.2 ± 0.9a	69.1 ± 15.7a	32.2 ± 4.3a	15.2 ± 2.5a

[a] Numbers followed by different letters in each column within each year were significantly different according to ANOVA and LSD Student's *t* tests ($\alpha \leq 0.05$)

Table 2 Parasitoid taxa and relative abundance reared or dissected from emerald ash borer (EAB), *Agrilus planipennis,* larvae from ash trees from study sites near Lansing, Michigan, USA

Year	Parasitoid taxa	No. individuals collected from different sites				Relative abundance ± SE (%)[c]	Parasitism of EAB larvae ± SE (%)[c,d]
		Burchfield	Central/Nancy Moore	Harris/ Legg	All sites combined	All sites combined	All sites combined
2009	*Tetrastichus planipennisi*	160	396	75	631	93.8 ± 0.93	0.75 ± 0.13
	Phasgonophora sulcata	0	18	5	23	3.4 ± 0.70	0.57 ± 0.11
	Atanycolus spp.	1	2	6	9	1.3 ± 0.44	0.22 ± 0.07
	Spathius spp.	6	0	0	6	0.9 ± 0.36	0.05 ± 0.03
	Balcha indica	0	1	0	1	0.1 ± 0.12	0.02 ± 0.02
	Others[a]	0	2	1	3	0.4 ± 0.24	0.07 ± 0.04
2010	*Tetrastichus planipennisi*	761	397	67	1225	58.1 ± 1.07	1.21 ± 0.17
	Atanycolus spp.	371	95	308	774	36.7 ± 1.05	19.4 ± 0.62
	Spathius spp.	14	0	36	50	2.4 ± 0.33	0.27 ± 0.08
	Phasgonophora sulcata	9	5	14	28	1.3 ± 0.24	0.69 ± 0.13
	Balcha indica	16	2	7	25	1.2 ± 0.23	0.61 ± 0.12
	Others[b]	1	1	3	5	0.2 ± 0.10	0.12 ± 0.05

[a] Including one individual of *Eurytomus* sp. and two individuals of *Dolichomitus* sp.

[b] Including three individuals of *Eupelmus* sp. and two individuals of *Eurytomus* sp.

[c] Standard errors are calculated using the formula: sqrt $[p * (1 - p)/n]$, where p = proportion and n = total number of parasitoids or EAB larvae observed

[d] Calculated as percentage of the total EAB larvae observed (4013 for 2009 and 4066 for 2010)

sites, but 25 individuals were collected in 2010 from our study sites. The relative abundance of this species increased from 0.1% to 1.2% from 2009 to 2010. Other solitary ectoparasitoids (*Eurytomus* sp., *Dolichomitus* sp. and *Eupelmus* sp.) were also collected from different study sites. However, their abundance was very low (<0.5% of all reared parasitoids) and resulted in <0.2% parasitism in both years.

Seasonal parasitism of EAB larvae in parasitoid-release and non-release control plots

Changes in larval parasitism (%) over season at the release and non-release plots (results combined for the three release sites) are summarized by parasitoid species or group (Figs. 1, 2, 3, 4, 5, and 6). Parasitism of EAB larvae varied significantly with sampling season for the three most abundant groups of parasitoid, *T. planipennisi* (Fig. 1) ($F = 4.06$, $df = 5, 85$; $P = 0.0024$), *Atanycolus* spp. (Fig. 2) ($F = 41.79$, $df = 5, 85$; $P < 0.0001$), and *P. sulcata* (Fig. 3) ($F = 3.55$, $df = 5, 85$; $P = 0.0058$), but not for the less abundant groups, including *Spathius* spp. (Fig. 4) ($F = 0.58, df = 5, 85; P = 0.7116$), *B. indica* (Fig. 5) ($F = 1.80$, df = 5, 85; $P = 0.1208$), and other less common species grouped (Fig. 6) ($F = 2.23$, $df = 5, 85$; $P = 0.0579$). No significant differences in parasitism were detected between the study sites or between the release and non-release (control) plots by parasitoid species or group ($P > 0.05$ for all tests).

For the introduced biological control agent *T. planipennisi*, a low level (1–5%) of parasitism of EAB larvae was consistently detected in the release plots from the summer of 2009 and into the fall of 2010, after the last field release in the summer of 2009 (Fig. 1). Low levels of EAB parasitism by *T. planipennisi* were also detected in one control plot immediately after the field releases in the summer of 2009, indicating that adults of *T. planipennisi* had dispersed >1 km to the control plots immediately following

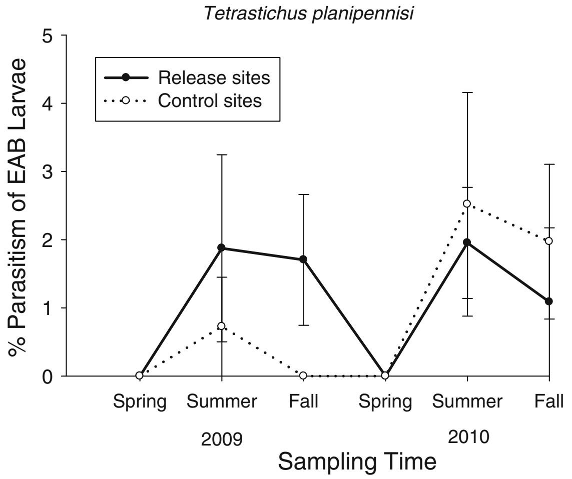

Fig. 1 Seasonal parasitism (±SE) of emerald ash borer (EAB), *Agrilus planipennis*, larvae in 2009 and 2010 by *T. planipennisi*, a gregarious endoparasitoid introduced from China. Mean parasitism and standard error were calculated across all the study sites ($n = 3$) for both parasitoid release and control treatments at each sampling time

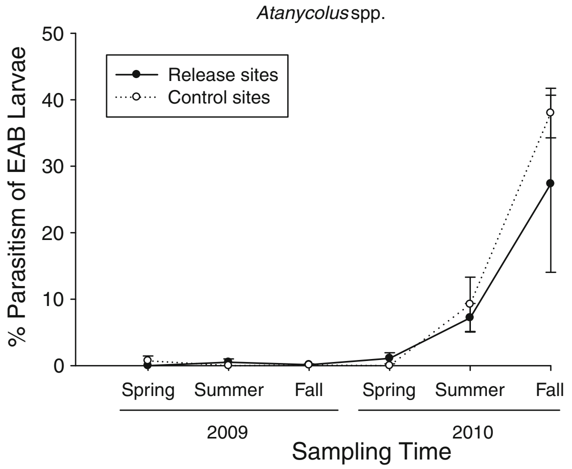

Fig. 2 Seasonal parasitism (±SE) of emerald ash borer (EAB), *Agrilus planipennis,* larvae in 2009 and 2010 by *Atanycolus* spp. (predominantly *A. cappaerti*), North American native solitary ectoparasitoids. Mean parasitism and standard error were calculated across all the study sites ($n = 3$) for both parasitoid release and control treatments at each sampling time

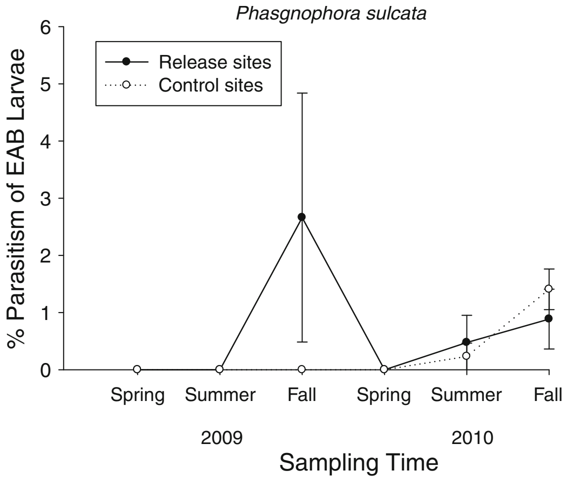

Fig. 3 Seasonal parasitism (±SE) of emerald ash borer (EAB), *Agrilus planipennis,* larvae in 2009 and 2010 by *P. sulcata*, a North American native, solitary endoparasitoid. Mean parasitism and standard error were calculated across all the study sites ($n = 3$) for both parasitoid release and control treatments at each sampling time

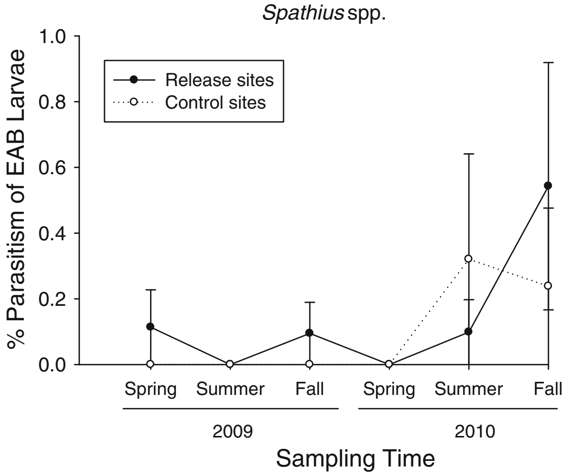

Fig. 4 Seasonal parasitism (±SE) of emerald ash borer (EAB), *Agrilus planipennis,* larvae in 2009 and 2010 by *Spathius* spp., primarily *S. floridanus,* a North American native, gregarious ectoparasitoid. Mean parasitism and standard error were calculated across all the study sites ($n = 3$) for both parasitoid release and control treatments at each sampling time

the first field release. The low level of EAB parasitism (2–4%) by *T. planipennisi* observed in the control (non-release) plots was comparable to that in the release plots after the spring of 2010.

Parasitism of EAB larvae by the native parasitoids *Atanycolus* spp. was low (<1%) until the spring of 2010, but increased to 19% by summer 2010. By the fall of 2010 *Atanycolus* spp. it increased another two-fold to 36% (Fig. 2). Percent parasitism by *P. sulcata* (Fig. 3), *Spathius* spp. (Fig. 4), *B. indica* (Fig. 5) and other-grouped parasitoids (Fig. 6) also varied with different sampling times. No significant seasonal changes were detected in these groups of parasitoids.

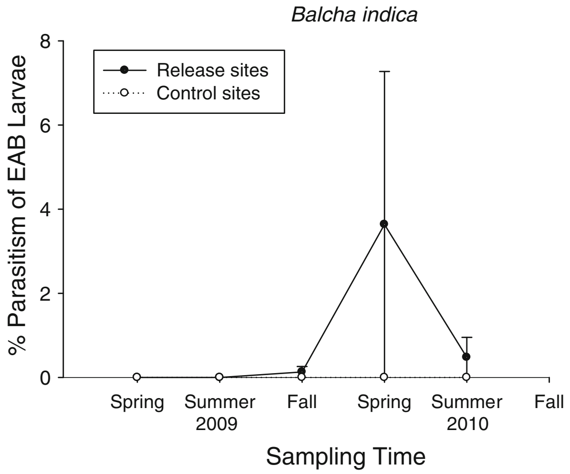

Fig. 5 Seasonal parasitism (±SE) of emerald ash borer (EAB), *Agrilus planipennis,* larvae by *B. indica*, an exotic parthenogenic, solitary ectoparasitoid native to southeast Asia. Mean parasitism and standard error were calculated across all the study sites ($n = 3$) for both parasitoid release and control treatments at each sampling time

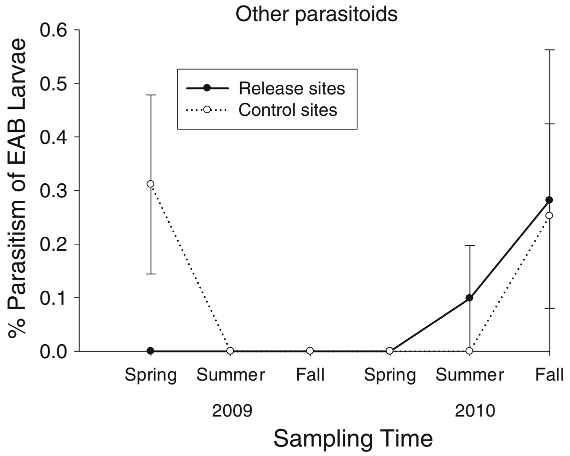

Fig. 6 Seasonal parasitism (±SE) of emerald ash borer (EAB), *Agrilus planipennis,* larvae in 2009 and 2010 by less abundant North American native species of hymenopteran parasitoids, including *Eurytomus* sp., *Dolichomitus* sp., and *Eupelmus* sp. Mean parasitism and standard error were calculated across all the study sites ($n = 3$) for both parasitoid release and control treatments at each sampling time

Discussion

During our two-year study following the introduction of two EAB larval parasitoids in forested areas of central Michigan, we found that EAB populations were heavily attacked by woodpeckers, undetermined biotic factors (such as pathogens and/or host plant resistance) and a diverse group of hymenopteran parasitoids, including the introduced biocontrol agent *T. planipennisi*. Population densities of EAB appeared to have been reduced by those mortality agents across different study sites from 87.1–126.5 in 2009 to 63.9–90.2 (larvae m^{-2} phloem) in 2010 (Table 1).

Throughout the study, woodpeckers consumed an average of 32–42% of the immature EAB stages in ash trees at our sites. These results are similar to those reported by Cappaert et al. (2005) and Lindell et al. (2008). Undetermined biotic factors killed 10–22% of the observed EAB larvae. Currently, it is not known how predation by woodpeckers and unknown sources of mortality including fungal pathogens (Liu ?tul?> and Bauer 2006) and host tree resistance mechanisms (Duan et al. 2010) interact with hymenopteran parasitoids and ultimately affect EAB population dynamics in Michigan. Kilham (1965) found that woodpeckers locate prey either with percussion causing them to move or with differential reverberation between an insect gallery and solid bark or wood. From our observations in Michigan, we found that some EAB galleries opened by woodpeckers contained empty *Atanycolus* spp. cocoons or dead *T. planipennis* larvae (JJD, unpublished data) or portions of cadavers with symptoms of fungal infection (LSB, unpublished data). Based on our current knowledge of prey-locating behaviors of woodpeckers and recent observations, it is reasonable to assume that woodpeckers may not discriminate between parasitized and non-parasitized EAB larvae. Thus, the avian predators may act as contemporaneous mortality factors with EAB larval parasitoids and pathogens to affect EAB population dynamics, causing significant underestimation of the actual rate of parasitoid attack (Elkinton et al. 1992).

It is known that emerald ash borer larvae are susceptible to fungal pathogens such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Liu and Bauer 2006, Castrillo et al. 2008) and also to host plant resistance (Duan et al. 2010). The reported levels of EAB larval mortality due to pathogens in Michigan were low (<5% of infestation rate) in the field (Bauer et al. 2004; Liu et al. 2007), and not associated with parasitism (Duan et al. 2010). High mortality levels due to tree resistance (10–41%) in young (1st–2nd instar) EAB larvae have been observed, resulting in growth of callus tissue (Duan et al. 2010). It is likely that undetermined biotic mortality factors such as host tree resistance may act before EAB larval parasitism, and thus negative interference with the hymenopteran parasitoids may be minimal. In addition, we also noted that the undetermined mortality increased sharply from 2009 to 2010 in tandem with a sharp increase in parasitism rate particularly by the native group of larval parasitoid *Atanycolus* spp. Therefore, it is likely that a substantial part of this undetermined mortality could be due to the unsuccessful parasitism by *Atanycolus* spp., which can paralyze host larvae without successfully parasitizing them.

Among the hymenopteran parasitoids associated with EAB populations, one introduced larval parasitoid, *T. planipennisi*, has successfully established a presence with a consistent low level of EAB larval parasitism (1–5%) at our study sites. Largely because of its gregarious life history, *Tetrastichus planipennisi* accounted for 93% of all the parasitoid individuals collected immediately after field release in 2009 and 58% one year later in 2010. In the first year of survey, we also found that adults of *T. planipennisi* appeared to be able to disperse >1 km to the control plots immediately following the field release. Future studies need to determine the rate of spread by *T. planipennisi* and the minimum distance required for setting up non-release control plots or between the two release points for biological control of EAB.

In contrast to *T. planipennisi*, the abundance of the native parasitoid *Atanycolus* spp. increased sharply from 2009 to 2010. *Atanycolus* spp. abundance accounted for approximately 1% of the parasitoid complex in 2009. In 2010, however, the prevalence of *Atanycolus* spp. increased to *ca*. 37%, resulting in an average increase in EAB parasitism rate from <0.5% in 2009 to 19% in 2010 by this parasitoid group. The sudden increase in the population of *Atanycolus* spp. in 2010 was likely a numerical response of this species to high densities of EAB at our study sites. Other groups of parasitoids such as *P. sulcata*, *Spathius* spp., *Balcha indica*, *Eupelmus* sp., and *Eurytomus* sp. were much less abundant than *T. planipennisi* and *Atanycolus* spp. and have not yet shown any numerical response to the EAB. Populations of the introduced biological control agent *S. agrili* in our study sites were still too low to allow any detection from the sampled EAB populations. This lack of detection of *S. agrili* could also be the result of small releases as ≈300 females were released at each study sites. More studies are needed to investigate if the introduced biological control agent *S. agrili* has become established in Michigan or more releases would be needed to facilitate its establishment.

A low prevalence of *Atanycolus* spp. has been reported attacking EAB larvae in other short-term studies of EAB (Bauer et al. 2004 in southeast Michigan; Duan et al. 2009 in western Pennsylvania; Kula et al. 2010 in northern Ohio). Cappaert and McCullough (2009), however, reported a high prevalence of *Atanycolus* spp. parasitism of EAB larvae in a southeast Michigan woodlot, where the majority of mature ash trees were dead. Apparently, by sampling the same field sites repeatedly over time, we captured the successional change in the species complex of parasitoid attacking EAB at these sites. Such dynamic changes in the prevalence and species diversity of EAB parasitoids will likely continue and will vary depending on the tree and *Agrilus* spp. from which these native parasitoids originated, the host range(s) of these parasitoids, the local EAB density, and degree of disturbance due to ash mortality.

The attack of EAB larvae by several hymenopteran parasitoids in Michigan (the first invaded region) is not surprising as EAB larvae are also attacked by several species of parasitoids in northeast Asia, the likely area of origin (Liu et al. 2003; Liu and Bauer 2006; Bray et al. 2011; Duan et al. 2011). In addition, there are many examples where a single insect host is attacked by a range of parasitoid species (Hawkins 1994; Memmott et al. 1994; Parry 1995). However, interspecific competition among such contemporaneously acting species is likely to affect their co-existence and may affect the establishment of the newly introduced biological control agents (e.g., Reitz 1996; Bogran et al. 2002; Hackett-Jones et al. 2009). How indigenous parasitoids such as *Atanycolus* spp. in North American will interact or compete with introduced biological control agents such as *T. planipennisi* and *S. agrili* should be further investigated.

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Author Biographies

Jian J. Duan is a research entomologist and lead scientist with the USDA Agricultural Research Service, Beneficial Insects Introduction Research Unit. His research focus is on biological control of invasive pests such as emerald ash borers, which involves (a) natural enemy exploration, importation, quarantine safety testing, and non-target risk assessment; (b) ecological and behavioral studies on host habitat/and host utilization patterns of introduced parasitoids; (c) development of spatial and temporal population dynamic models to predict the colonization, establishment, and distribution of imported natural enemies in their region of new introduction.

Leah S. Bauer is a research entomologist for USDA Forest Service. In 2002, she began studying the emerald ash borer, focusing on its management with microbial and biological controls.

Kristopher J. Abell is a postdoctoral research associate at University of Massachusetts. His research interest is in biological control of agricultural and forest insect pests, and has been working on biological control of emerald ash borer since 2009.

Roy van Driesche is a full professor at the University of Massachusetts. His research focus is on classical biological control of invasive insects, especially those affecting forests and other natural areas.