# Establishment and Abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: Potential for Success in Classical Biocontrol of the Invasive Emerald Ash Borer (Coleoptera: Buprestidae)

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**ABSTRACT** *Tetrastichus planipennisi* Yang is a gregarious larval endoparasitoid native to China and has been introduced to the United States since 2007 for classical biological control of the invasive emerald ash borer, *Agrilus planipennis* Fairmaire, an exotic beetle responsible for widespread ash mortality. Between 2007–2010, *T. planipennisi* adults (3,311–4,597 females and ≈1,500 males per site) were released into each of six forest sites in three counties (Ingham, Gratiot, and Shiawassee) of southern Michigan. By the fall of 2012, the proportion of sampled trees with one or more broods of *T. planipennisi* increased to 92 and 83% in the parasitoid-release and control plots, respectively, from 33 and 4% in the first year after parasitoid releases (2009 fall for Ingham county sites and 2010 for other sites). Similarly, the mean number of *T. planipennisi* broods observed from sampled trees increased from less than one brood per tree in the first year after parasitoid releases to 2.46 (at control plots) to 3.08 (at release plots) broods by the fall of 2012. The rates of emerald ash borer larval parasitism by *T. planipennisi* also increased from 1.2% in the first year after parasitoid releases to 21.2% in the parasitoid-release plots, and from 0.2 to 12.8% for the control plots by the fall of 2012. These results demonstrate that *T. planipennisi* is established in southern Michigan and that its populations are increasing and expanding. This suggests that *T. planipennisi* will likely play a critical role in suppressing emerald ash borer populations in Michigan.

**KEY WORDS** natural enemy introduction, invasive, wood borers, parasitoid release and establishment

Classical biological control, the introduction, and establishment of natural enemies from the native range of target pests, can be an extremely cost-effective, sustainable, and environmentally benign tool for management of agricultural and forest pests. With the increasing number of exotic pests invading forests of the United States in recent decades (Aukema et al. 2010), it is imperative that biological control be given priority as a management tool for use against these invasive pests.

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a relatively new invasive forest pest that has killed tens of millions of ash (*Fraxinus*) trees throughout the eastern United States since it was first detected in 2002 in Michigan and Ontario, Canada (Haack et al. 2002, Cappaert et al. 2005, Poland and McCullough 2006, Kovacs et al. 2010, Michigan State University 2012, Canadian Food Inspection Agency 2012). Research on natural enemies of the emerald ash borer was initiated shortly after its discovery and resulted in a classical biological control program using three hymenopteran parasitoids native to northern China (Bauer et al. 2007, 2008), where United States emerald ash borer populations likely originated (Bray et al. 2011). These three biocontrol agents include an egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al. 2005), a larval ectoparasitoid, *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al. 2005), and a larval endoparasitoid, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Yang et al. 2006).

After research on the biology, laboratory rearing, and host specificity of the three parasitoid species was completed in 2007, federal and state regulatory agencies approved their environmental release in Michigan (Federal Register 2007, Bauer et al. 2008). Parasitoid releases expanded to Ohio and Indiana in 2008; Maryland and Illinois in 2009; West Virginia, Kentucky, and

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Minnesota in 2010; and New York, Pennsylvania, Virginia, and Wisconsin by 2011 (Bauer et al. 2012, MapBioControl.org 2012). Although one or more of these parasitoids are reported to have established at release sites in Michigan, Maryland, Ohio, Indiana, and Illinois (Bauer et al. 2010, 2011; Gould et al. 2010; Duan et al. 2010, 2012a), levels of parasitism were considerably lower (<5%) than levels (12–73%) reported from China (Liu et al. 2003, 2007).

Among these biocontrol agents, *T. planipennisi* appears to have a narrower host range than *O. agrili* and *S. agrili* (Bauer et al. 2007, Federal Register 2007). Recent studies (Duan et al. 2011, Duan and Oppel 2012) indicate that this parasitoid has a short generation time (≈4 wk), high reproductive potential (lifetime realized fecundity averaging 57 progeny and maximum 108 progeny per female) and a highly female-biased sex ratio. In addition, field surveys in China also showed that *T. planipennisi* is a dominant species of natural enemy associated with emerald ash borers in its native range, northeast China (Liu et al. 2003, 2007). Because of these characteristics, *T. planipennisi* is considered to have good potential to be an effective biocontrol agent against emerald ash borers (Bauer et al. 2007, 2008; Duan et al. 2011).

In the current study, we determined establishment and quantified changes in abundance of *T. planipennisi* for multiple years after its environmental release at six study sites, each comprised of a release and no-release control plot, in southern Michigan. In addition to parasitism by *T. planipennisi,* we also documented emerald ash borer larval mortality caused by woodpeckers, putative host tree resistance, diseases, and other larval parasitoids across the study sites.

## Materials and Methods

**Study Sites.** The study was conducted in six, mixed hardwood bottomland forests (sites) located in three southern Michigan counties: Ingham, Gratiot, and Shiawassee (Fig. 1). The exact latitude and longitude for each of the six sites can be found in Duan et al. (2010) and MapBiocontrol (2013). Three study sites were located in Ingham Co. in 2007–2008: two groups of adjacent Meridian Township parks: 1) Central Park and Nancy Moore Park (CP), 2) Legg Park–Harris Nature Center (LP), and one county park, 3) William M. Burchfield Park (BF). CP and LP were ≈5 km from each other and 32 km away from BF. Three additional study sites, located on Michigan's Department of Natural Resources lands, were established in 2009–2010. Two of the sites in Gratiot Co. were located ≈10 km apart: 4) Gratiot-Saginaw State Game Area (GSW) and 5) Maple River State Game Area (MRE). The remaining site was located in Shiawassee Co.: 6) Rose Lake State Wildlife Area (RL), which was ≈60 km from the MRE and 15 km from CP.

Each study site was comprised of two adjacent treatment plots, each ≥10 ha and separated from each other by 1–6 km. The plots were randomly selected for either parasitoid-release or nonrelease control treatment. All study sites were located in early successional, secondary-growth northern deciduous forest, initially dominated by green ash (*F. pennsylvanica* Marshall). Less abundant tree species included white ash (*F. americana* L.), black ash (*F. nigra* Marshall), maples (*Acer*), oak (*Quercus*), black cherry (*Prunus serotina* Ehrh), poplar (*Populus*), black walnut (*Juglans nigra* L.), basswood (*Tilia americana* L.), and some pine (*Pinus*). Although there were notable differences in tree species composition, abundance, tree basal area, and tree diameter at breast height (DBH; ≈1.5 m above the ground) among the six study sites, these characteristics were similar between the parasitoid-release and control plots within a site. Symptoms of emerald ash borer infestation (reduced canopy, woodpecker attack, and epicormic growth) were observed in all study sites, particularly on mature large ash trees at the time of initiating the study (2007–2008 for Ingham Co. sites and 2009–2010 for Gratiot and Shiawassee Co. sites). Based on these indirect estimates, emerald ash borer populations were at or near peak densities when *T. planipennisi* was released.

**Parasitoid Rearing and Field Releases.** All adults of *T. planipennisi* released for this study were progeny (generations $F_4$ to $F_{30}$) of parasitoids originally collected from northeast China (Liu et al. 2007, Duan et al. 2011). *T. planipennisi* was reared on field-collected emerald ash borer larvae at the USDA Forest Service–Northern Research Station (USDA FS–NRS) (East Lansing, MI), the USDA–APHIS (Animal and Plant Health Inspection Service) Emerald Ash Borer Biocontrol Laboratory (Brighton, MI), and the USDA–ARS (Agriculture Research Services) Beneficial Insect Introduction Research Unit (USDA-ARS–BIIR) (Newark, DE), according to methods described in Ulyshen et al. (2010) and Duan and Oppel (2012).

Before the parasitoids were released, female and male *T. planipennisi* were held together inside rearing cages for at least 3 d to allow for mating (Duan et al. 2011). The timing of parasitoid releases and numbers of adult females and males released at each of the six study sites are summarized in Table 1. Briefly, the first releases of *T. planipennisi* adults were made at the three study sites in Ingham Co.; however, only small quantities were available for release during fall 2007 at CP (671 females), and summer through fall 2008 at CP, LP, and BF (111–203 females per site). In April 2009, a survey of emerald ash borer-infested ash trees at both release and control plots of the three Ingham Co. sites failed to recover *T. planipennisi,* therefore, an additional 3,200 females were released at each of these release plots between May and September 2009. Releases of *T. planipennisi* for the other three sites in Gratiot and Shiawassee counties were made in large quantities (3,828–3,897 females per site) between June and September 2010, approximately 1 yr later than for the Ingham Co. sites. Major releases of *T. planipennisi* in all study sites were staggered in time, with some adults released every 1 to 3 wk from May through September of 2009 (for the Ingham Co. sites) and 2010 (for the Gratiot and Shiawassee Co. sites). *T. planipennisi* adults were released onto the lower 2 m

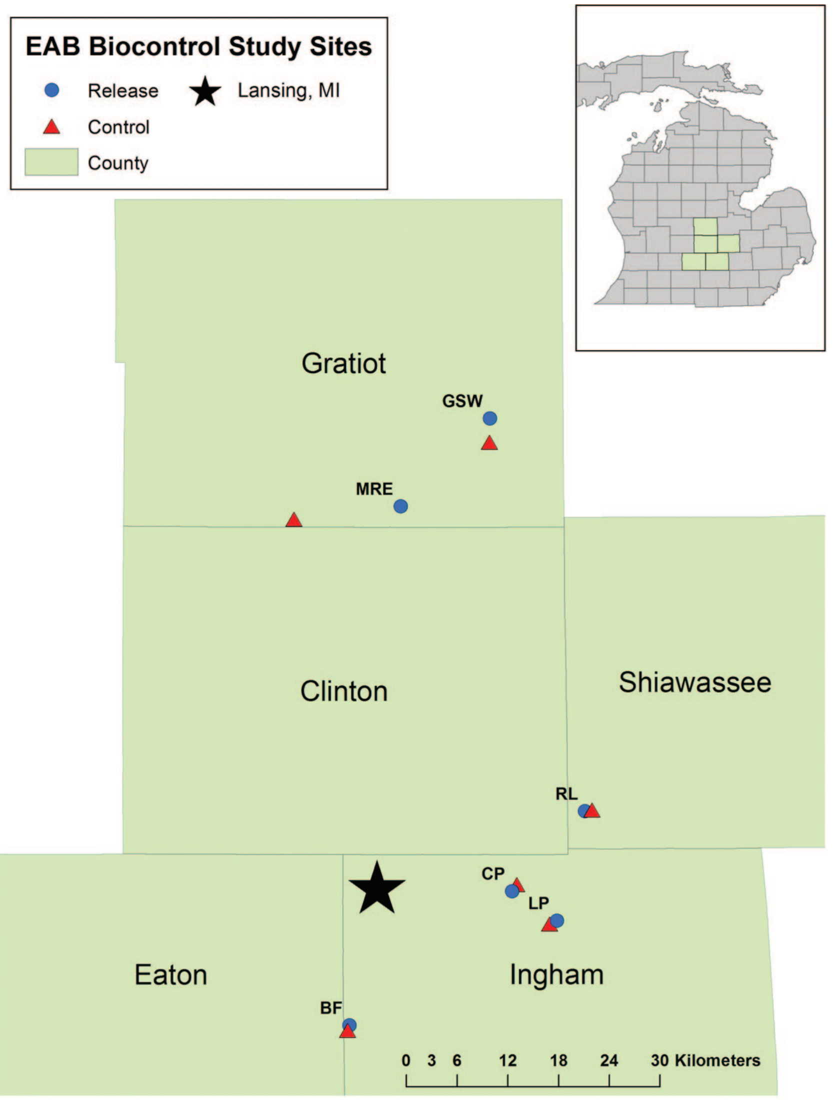


**Fig. 1.** Map of Michigan showing the location of the six study sites and treatment plots. The six study sites are: BF, Williams F. Burchfield Park; CP, Central Park and Nancy Moore Park; LP, Legg Park and Harris Natural Center; GSW, Gratiot-Saginaw State Game Area; MRE, Maple River State Game Area; RL, Rose Lake State Wildlife Area. (Online figure in color.)

of trunk of 4–10 ash trees distributed within a radius of 100–300 m from the center of each release plot.

**Sampling Procedures.** To investigate the establishment and abundance of *T. planipennisi* and other emerald ash borer larval mortality factors, we initiated a sampling program in the spring (25 April through 12 May) of 2009 for the Ingham Co. sites (after the initial parasitoid releases in 2007–2008) and in the spring (20–25 April) of 2011 for the Gratiot and Shiawassee Co. sites (after the initial parasitoid releases in 2009–2010). Because of major flooding at one of the Gratiot Co. sites (MRE) in spring 2011, no samples were taken from this site until spring (22–29 April) of 2012. Otherwise, sampling at each study site was conducted every year after the initial parasitoid releases, either in the fall or early spring. Previous studies (Duan et al. 2010, 2012a) showed that symptoms of emerald ash borer larval parasitism by *T. planipennisi* are most apparent in the fall when *T. planipennisi* larvae normally emerge from host larvae for overwintering, or in the early spring when overwintering *T. planipennisi* are still in the host gallery as larvae, pupae, or pharate adults. Because of the cold conditions found from late fall to early spring in Michigan, we assumed that populations of both emerald ash borer and its associated parasitoids were relatively static during this period, and thus we combined samples of early spring of a given year with the fall samples of the previous year for data analyses and presentation. Although rates of woodpecker predation of emerald ash borer larvae may change throughout the year, we found no evidence that woodpeckers preferred emerald ash

**Table 1. Time and numbers of *T. planipennisi* adults released at study sites in southern Michigan**

| County | Study sites[a] | Release time (year: month) | No. of releases | No. of females released | No. of males released |
|---|---|---|---|---|---|
| Ingham | BF | 2008: July | 1 | 111 | 51 |
| | | 2009: May–Sept. | 9 | 3,200 | 1,134 |
| | CP | 2007: Sept.–Oct. | 2 | 671 | 246 |
| | | 2008: June | 1 | 147 | 30 |
| | | 2009: June–Oct. | 10 | 3,200 | 1,388 |
| | LP | 2008: Sept.–Oct. | 5 | 203 | 63 |
| | | 2009: May–Sept. | 10 | 3,200 | 1,235 |
| Gratiot | GSW | 2009: Aug.–Sept. | 8 | 700 | 8 |
| | | 2010: June–Sept. | 7 | 3,897 | 1,569 |
| | MRE | 2010: June–Sept. | 6 | 3,828 | 1,437 |
| Shiawassee | RL | 2010: July–Sept. | 8 | 3,879 | 1,165 |

[a] BF, Burchfield Park; CP, Central Park-Nancy Moore Parks; LP, Legg Park-Harris Nature Center; GSW, Gratiot Saginaw State Game Area; MRE, Maple River State Game Area; RL, Rose Lake State Wildlife Area.

borer larvae parasitized by *T. planipennisi* to those unparasitized.

At each sample time, two to six live ash trees with apparent symptoms of emerald ash borer infestation (e.g., woodpecker feeding, epicormic growth) from the release and control plots at each study site were sampled using procedures described in Duan et al. (2012a). For sampling, ash trees were felled using a bow or chain saw. Each tree including the main trunk and branches >3 cm in diameter were debarked with a drawknife and examined for the presence of immature stages of emerald ash borers and associated parasitoids. Parasitism of emerald ash borer larvae by *T. planipennisi* was scored in the field based on the presence of visible stages of the parasitoid larvae (emerged larvae, pupae, meconium, or adults) in host galleries. In addition, emerald ash borer larvae were also collected and returned to either the USDA-FS–NRS laboratory or the USDA-ARS–BIIR quarantine facility for rearing or dissection to detect other cases of parasitism.

In addition to parasitism by *T. planipennisi,* we recorded four other categories of mortality associated with emerald ash borer immature stages: 1) parasitism by other hymenopteran parasitoids, primarily the native hymenopterans *Atanycolus* spp. and *Phasgonophora sulcata* Westwood; 2) woodpecker predation; 3) mortality because of host tree resistance, visible as small first- or second-instar larvae or galleries overgrown with tree callus tissue; and 4) mortality from unknown diseases, which often occurred with larger emerald ash borer larvae (third instars and older), with cadavers most often associated with fungi or putrification (Liu and Bauer 2006; Duan et al. 2010, 2012a).

To calculate parasitism rates of emerald ash borer larvae by *T. planipennisi*, we excluded emerald ash borer larvae preyed upon by woodpeckers and those apparently killed by host tree resistance. 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. Based on this finding and previous observations by Duan et al. (2010, 2012a), it is reasonable to assume that woodpeckers locate gallery voids as they do not seem to discriminate between parasitized and nonparasitized emerald ash borer larvae. Thus, the exclusion of this portion of mortality does not affect estimates of *T. planipennisi* parasitism. Exclusion of emerald ash borer larvae killed by putative host tree resistance was justified mainly because this occurs during the first- and second-larval instar (Duan et al. 2010, 2012b), host stages rarely parasitized by *T. planipennisi* (Liu et al. 2007, Ulyshen et al. 2010). As we currently have no information on interactions between *T. planipennisi* and diseased emerald ash borer larvae (third instars or older), we did not exclude this mortality category from the sample size used for calculating *T. planipennisi* parasitism rates.

**Data Analysis.** To evaluate the establishment of *T. planipennisi* at different study sites, we used a multiple logistic regression model to analyze the probability that a sampled tree would have one or more broods of *T. planipennisi* in relation to different number of years after parasitoid release, with parasitoid-release treatments and study sites as codependent variables. We used a general linear (analysis of variance [ANOVA]) model to analyze the abundance (mean number of broods observed per tree) of *T. planipennisi* in relation to parasitoid release treatments, study sites, and years after the last parasitoid release. To assess the impact of *T. planipennisi* populations on the level of emerald ash borer mortality, we first calculated the parasitism rate for each sampled tree based on the number of emerald ash borer larvae associated with immature and/or mature (adult) stages of *T. planipennisi,* and then used the general linear (ANOVA) model to analyze the relationship between rates of parasitism in both release and control plots, the number of years after the last parasitoid release, and the study site. Before analysis, the mean number of *T. planipennisi* broods observed per tree was transformed using a square root function to normalize the distribution, while data on parasitism rates were transformed using an arcsine square root function.

In addition, we used the general linear model (ANOVA) to evaluate differences in DBH, emerald ash borer density, and emerald ash borer mortality caused by woodpeckers, putative plant resistance, diseases, and other parasitoids between parasitoid-release and control plots, as well as among different study sites and sampling times. Emerald ash borer density was calculated based on the total number of all emerald ash borer stages observed and the phloem area of each sampled tree, which was estimated using the method described by McCullough and Siegert (2007). All statistical analyses were carried out with JPM 10.01 statistical software (SAS Institute 2012).

## Results

**Emerald Ash Borer Density and Mortality Caused by Biotic Factors Other Than *T. planipennisi*.** Although the average DBH (centimeters) of sampled ash trees varied significantly among the six study sites ($F = 5.57$; df = 5, 173; $P < 0.0001$) and sampling time ($F = 10.74$; df = 8, 173; $P < 0.0001$), there was no significant

**Table 2. Mean (± SE) tree DBH, emerald ash borer larval density, and mortality across the six sites caused by woodpeckers, host tree resistance, undetermined diseases, and parasitoids other than *T. planipennisi* in release and control plots in southern Michigan**

| Year (fall) | Treatment plots | No. trees sampled | DBH (cm) of sampled trees | Emerald ash borer density ($n$)/m$^2$ phloem | % emerald ash borer killed by woodpeckers | % emerald ash borer killed by trees | % emerald ash borer killed by diseases | % emerald ash borer killed by parasitoids |
|---|---|---|---|---|---|---|---|---|
| 2008 | Release | 15 | 12.1 ± 1.3 | 51.1 ± 8.2 | 49.3 ± 5.5 | 1.8 ± 0.7 | 3.9 ± 1.0 | 0.12 ± 0.1 |
| | Control | 14 | 10.9 ± 1.1 | 58.1 ± 6.7 | 52.3 ± 5.1 | 0.19 ± 0.1 | 1.3 ± 0.5 | 0.41 ± 0.2 |
| 2009 | Release | 20 | 9.3 ± 0.7 | 63.8 ± 12.6 | 27.1 ± 5.1 | 15.2 ± 4.5 | 2.2 ± 0.9 | 1.2 ± 0.5 |
| | Control | 21 | 8.8 ± 0.8 | 47.9 ± 11.3 | 34.2 ± 5.7 | 13.6 ± 3.5 | 2.1 ± 0.9 | 0.01 ± 0.01 |
| 2010 | Release | 23 | 10.4 ± 0.4 | 61.2 ± 8.1 | 41.1 ± 2.8 | 13.1 ± 2.5 | 9.0 ± 2.0 | 14.2 ± 2.8 |
| | Control | 22 | 10.5 ± 0.5 | 74.7 ± 10.5 | 34.6 ± 3.4 | 12.1 ± 2.5 | 10.6 ± 1.6 | 18.2 ± 2.7 |
| 2011 | Release | 12 | 11.5 ± 0.6 | 56.6 ± 7.7 | 36.8 ± 3.5 | 12.9 ± 3.5 | 2.3 ± 0.8 | 10.2 ± 2.4 |
| | Control | 12 | 11.1 ± 0.8 | 63.1 ± 7.4 | 34.9 ± 2.4 | 9.3 ± 2.2 | 2.7 ± 0.7 | 11.1 ± 1.9 |
| 2012 | Release | 24 | 8.9 ± 0.4 | 42.7 ± 7.7 | 56.6 ± 3.0 | 4.7 ± 1.1 | 2.5 ± 0.7 | 10.7 ± 2.0 |
| | Control | 24 | 8.7 ± 0.4 | 36.3 ± 3.8 | 56.0 ± 3.1 | 4.5 ± 0.8 | 1.7 ± 0.5 | 10.6 ± 1.8 |

difference ($F = 1.67$; df = 1, 118; $P = 0.1928$) in the mean (±SE) DBH of sampled trees between the parasitoid-release (10.2 ± 0.3 cm) and control (10.8 ± 0.3 cm) plots. There were no significant differences in emerald ash borer densities and mortality rates from woodpecker predation, putative tree resistance, unidentified diseases, and other (mostly North American native parasitoids) between parasitoid-release and control plots during the 5-yr study periods (All ANOVA tests $P > 0.05$). Across the six study sites, mean emerald ash borer density (number of immature stages per square meter of phloem) ranged from 42.7 to 63.8 for the parasitoid-release plots and 36.3–74.7 for the control plots throughout the 5-yr study period (Table 2). Approximately 27–57% and 34–56% of immature emerald ash borer stages (third instar or later stages) were preyed upon by woodpeckers during the 5-yr study period in the parasitoid-release and control plots, respectively (Table 2). Putative host tree resistance killed ≈1.8–15% and 0.2–14% of young emerald ash borer larvae (first and second instars) in the parasitoid-release and control plots, respectively. Unidentified diseases killed 2.2–9.0% in the parasitoid-release plots and 1.3–11% of emerald ash borer larvae (third or later instars) in the control plots. While <2% of emerald ash borer larvae were parasitized by other parasitoids in both parasitoid-release and control plots in 2008 and 2009, parasitism by this group of parasitoids (primarily North American native *Atanycolus* spp. and *P. sulcata*) increased to ≈14% in parasitoid-release plots and 18% in control plots by 2010 (fall).

**Recovery and Abundance of *T. planipennisi* in Sampled Ash Trees.** While no emerald ash borer larval parasitism by *T. planipennisi* was observed in ash trees sampled from either parasitoid-release or control plots in 2008 (sampled in spring of 2009) before the last (2009) parasitoid releases (for the Ingham Co. sites only), 33% of sampled ash trees in release plot ($n = 24$) and 4% in control plot ($n = 25$) were observed with one or more broods of *T. planipennisi* in the fall of 2009 (Fig. 2). By the fall of 2012, approximately 4 yr after the parasitoid releases (for the Ingham Co. sites only), the proportion of sampled ash trees with one or more broods of *T. planipennisi* in parasitoid release and control plots increased to 92% ($n = 12$) and 83% ($n =$

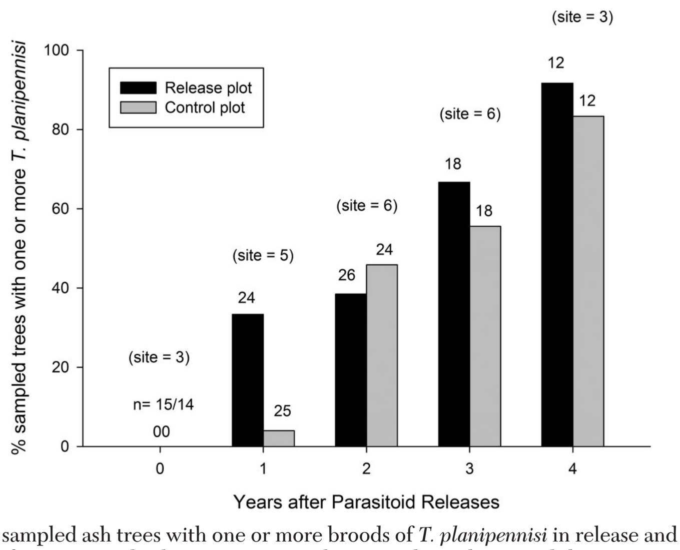


**Fig. 2.** Percentage of sampled ash trees with one or more broods of *T. planipennisi* in release and control plots across the six study sites each year after parasitoid releases were complete. Numbers above each bar represent total number of trees ($n$) sampled and sites included (in parenthesis). Zeros represent no observation of *T. planipennisi* on sampled trees. On the x-axis, year zero begins in 2008.

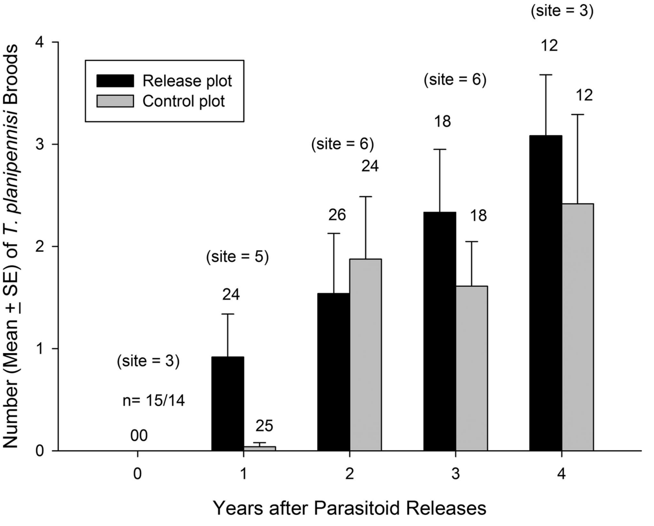


**Fig. 3.** Abundance (number) of *T. planipennisi* broods observed per sampled ash tree in both release and control plots across the six study sites each year after parasitoid releases were complete. Numbers above each bar represent total number of trees (*n*) sampled and sites included (in parenthesis). Zeros represent no observation of *T. planipennisi* broods. On the x-axis, year zero begins in 2008.

12), respectively (Fig. 2). Normal logistical regression analysis showed that the probability of recovering one or more broods of *T. planipennisi* from a sampled tree was significantly affected by the number of years after major parasitoid releases (Likelihood ratio $\chi^2$ = 68.679; df = 1; $P < 0.0002$) and study sites (Likelihood ratio $\chi^2$ = 13.4809; df = 1; $P$ = 0.0167), but not significantly by plot type (parasitoid-release vs. control) (Likelihood ratio $\chi^2$ = 2.5227; df = 1; $P$ = 0.1122), indicating that *T. planipennisi* quickly dispersed from the release plots to the nearby control plots in all study sites, and thus minimized the effect of parasitoid-release treatments.

A similar pattern was observed for the abundance of *T. planipennisi* (number of broods per tree) from both release and control plots at different sampling times after the major parasitoid releases at the six study sites (Fig. 3). The mean number of *T. planipennisi* broods observed from a sampled tree increased from less than one brood at the first year (2009 fall for Ingham sites and 2010 for other sites) after parasitoid releases to 2.46–3.08 broods (for both control and release plots) in the fourth year (fall 2012) after parasitoid releases (only from Ingham sites). ANOVA indicated that time after parasitoid release had a highly significant effect on the mean number of *T. planipennisi* broods observed per sampled tree ($F$ = 46.5553; df = 1, 142; $P <$ 0.0001). While the mean number of *T. planipennisi* broods observed on an ash tree varied significantly among the different study sites ($F$ = 2.5486; df = 5, 142; $P$ = 0.0295), there were no significant differences in parasitoid abundance between the release and control plots.

**Parasitism of Emerald Ash Borer Larvae by *T. planipennisi*.** Figure 4 summarizes percent parasitism of emerald ash borer larvae by *T. planipennisi* in the parasitoid-release and control plots for each of the six study sites at various times of sampling after parasitoid releases. No parasitism of emerald ash borer larvae by *T. planipennisi* was observed in 2008 (sampled in spring of 2009) from either the parasitoid-release or control plots in the three Ingham Co. sites (Fig. 4A–C). Low rates (<2%) of parasitism by *T. planipennisi* were observed in 2009 (sampled in the fall of 2009 or spring of 2010) from all parasitoid release plots. In contrast, a low rate (<2%) of parasitism was found at one of the control plots and no parasitism by *T. planipennisi* was found in the other control plots in 2009. Thereafter, parasitism by *T. planipennisi* increased annually and reached the highest value in 2012 (fall) samples, ranging from 14.7 to 28.6% in parasitoid-release plots and 2.9–27.7% in control plots. For the three study sites set up later in Gratiot and Shiawassee Co. (Fig. 4D–F), emerald ash borer larval parasitism by *T. planipennisi* by 2012 (fall) ranged from 5.2 to 17.4% in release plots and 6.0–10.5% in control plots.

When sampling time for each study site were readjusted to times (years) after parasitoid releases, rates of parasitism by *T. planipennisi* increased sharply in both parasitoid release and control plots after the last field releases (Fig. 5). Rates of emerald ash borer larval parasitism increased from 1.2% (at the first year of parasitoid releases) to 21.2% for the parasitoid release plot at the fourth year after parasitoid releases, and from 0.2 to 12.8% for control plots. Multiple regression analysis showed that time (year) after parasitoid releases had a highly significant effect on the rate of parasitism by *T. planipennisi* ($F$ = 30.7994; df = 4, 142; $P <$ 0.0001). While there were no significant differences in parasitism rate of emerald ash borer larvae by *T. planipennisi* among different study sites ($F$ = 1.6664; df = 5, 142; $P$ = 0.1452), there was a

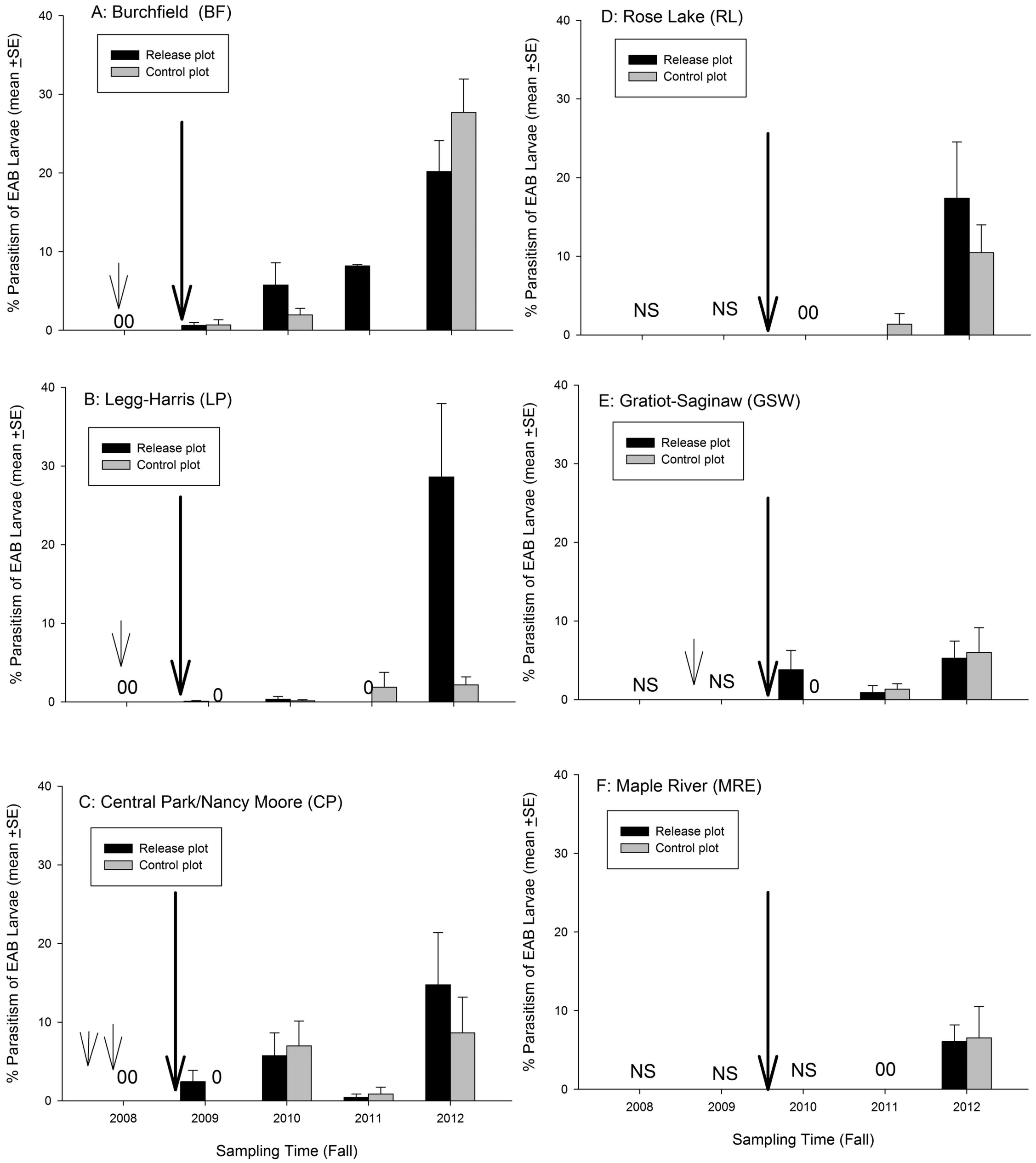


**Fig. 4.** Percent parasitism of emerald ash borer (EAB) larvae by *T. planipennisi* in both release and control plots at each of the six study site at various sampling years after parasitoid releases. In Fig. 4D–F (NS, "not sampled" as these study sites were not started until 2009 or 2010) or sampling was not done because of flooding (2010 in Fig. 4 F). Zeros represent no observation of *T. planipennisi* parasitism. Small arrows indicate the first year of *T. planipennisi* releases (low numbers) and large arrows indicate the major parasitoid releases (high numbers).

significant difference between parasitoid release and control plots ($F = 4.0348$; df = 1, 142; $P = 0.0461$).

## Discussion

Three to 4 yr after major field releases of *T. planipennisi* in emerald ash borer-infested ash stands in southern Michigan (between 2007–2009 in Ingham Co. sites and 2009–2010 in Gratiot and Shiawassee Co. sites), this biocontrol agent became established and common on emerald ash borer-infested ash trees in both release and control plots at all six study sites in southern Michigan. Approximately 4 yr after *T. planipennisi* releases began, 92% of sampled emerald ash borer-infested ash trees contained at least one brood of *T. planipennisi,* indicating the widespread dispersal

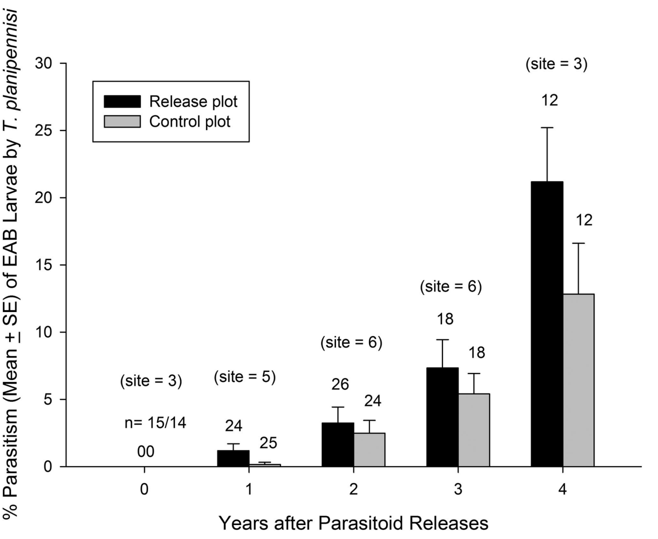


**Fig. 5.** Emerald ash borer (EAB) larval parasitism (%) by *T. planipennisi* in release and control plots across the six study sites in relation to time (years) after the last parasitoid releases. Numbers above each bar represent total number of trees (*n*) sampled and sites included (in parenthesis). Zeros represent no observation of *T. planipennisi* parasitism. On the x-axis, year zero begins in 2008.

of this parasitoid in our study sites after environmental releases. During that period, the mean number of *T. planipennisi* broods increased from about one brood per tree to three broods per tree, and emerald ash borer larval parasitism by *T. planipennisi* reached ≈21% in the release plots and ≈13% in the control plots. These results show that *T. planipennisi* established an increasing population in southern Michigan and is likely to play a critical role in suppressing emerald ash borer populations in the area.

Currently, knowledge of the spread and dispersal of *T. planipennisi* adults is limited, although early observations of *T. planipennisi* at our three Ingham Co. control plots 1 yr after field release indicated *T. planipennisi* spread at least 1 km/yr. At the site in Gratiot Co. (GSW) where the distance between release and control plots was almost 3 km, *T. planipennisi* was recovered in the control plot in 2010, 1 yr after its initial release in the area indicating that the spread rate may be higher. A higher *T. planipennisi* spread rate is also supported by evidence from incidental recoveries in southern Michigan. For example, *T. planipennisi* was recovered 16.4 km from the nearest release site made 3 yr earlier suggesting it may have spread >5 km per year (L.S.B. and J.P.L., unpublished data). Future studies on the spread and dispersal patterns of *T. planipennisi* in newly released areas would help design release strategies that optimize establishment of this species and increase its efficacy in controlling emerald ash borer populations.

During the course of this study, the mean DBH of sampled ash trees ranged from 8.7 to 12.1 cm because emerald ash borers killed the larger ash trees at our sites within 2 yr, and most surviving ash trees were <12 cm DBH. Earlier emerald ash borer parasitoid surveys in northeast China (Liu et al. 2003) and the Russian Far East (Duan et al. 2012b) report that *T. planipennisi* is indeed more prevalent in smaller diameter ash trees. The reason for this apparent bias was elucidated in a recent field experiment that shows *T. planipennisi* rarely parasitized emerald ash borer larvae in larger, thick-barked trees (>3.2 mm thick bark, typical of trees with >12 cm DBH) because of its relatively short ovipositor (average 2–2.5 mm) (Abell et al. 2012). The thick bark of large diameter ash trees provides a refuge for emerald ash borer larvae from attack by *T. planipennisi* (Abell et al. 2012). Abell et al. (2012) further suggests that *T. planipennisi* will be more effective in stands with younger trees (<12-cm DBH) such as in our study sites, or in natural ash regeneration found in some emerald ash borer-affected stands. To successfully control emerald ash borers on both small and larger ash trees, other emerald ash borer parasitoids from its native range with longer ovipositors, such as *Spathius galinae* Belokobylskij, should also be evaluated for release in emerald ash borer-invaded areas in North America (Belokobylskij et al. 2012, Duan et al. 2012b). *S. galinae* has a longer ovipositor (4–5 mm) than the previously introduced congener *S. agrili* (3–4 mm), and is currently being evaluated for potential releases in the United States (J.J.D., unpublished data).

Besides parasitism by *T. planipennisi,* emerald ash borer larvae also suffered heavy losses from other biotic factors including woodpeckers, putative plant resistance, diseases, and other larval parasitoids. Among the other mortality factors detected across our study sites, woodpeckers were the most abundant during the entire study period and across different study sites, removing up to 57% of older immature emerald ash borer stages (fourth instar to pupae) from feeding galleries and/or pupal chambers. Putative tree resistance killed up to 15% of younger larvae and unknown diseases caused similar levels of mortality of larger larvae throughout the study. Although <2% of emerald ash borer larvae across different study sites were parasitized by other parasitoids (primarily *Atanycolus* spp. and *P. sulcata*) before 2009, parasitism by these native parasitoids increased sharply to 18% in some of our plots by the following year. This increase in parasitism of emerald ash borer larvae by native parasitoids across our study sites was most likely the result of numerical responses of those native species to high emerald ash borer densities in our study sites (Duan et al. 2012a). How these other mortality factors, particularly the North American native parasitoids such as *Atanycolus* spp. that have longer ovipositors, interact with the newly introduced *T. planipennisi* in suppressing emerald ash borer populations are worthy of future research, and will be presented separately in future publications.

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