Forest Ecology and Management

**Emerald ash borer biocontrol in ash saplings: the potential for early stage recovery of
North American ash trees**

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ABSTRACT: In many parts of North America, ash (*Fraxinus*) stands have been reduced by the emerald ash borer (*Agrilus planipennis*) invasion to a few surviving mature trees, saplings, basal sprouts, and seedlings. Without a soil seed bank for *Fraxinus* spp., tree recovery will require survival and maturation of these younger cohorts to reproductive age. Here we report and analyze the population dynamics of emerald ash borer and its associated natural enemies in ash saplings (2.5 – 5.8 cm DBH) in six deciduous forest stands in southern Michigan. At these sites, the outbreak population of the pest collapsed during the study, and a biocontrol agent introduced from China, the larval parasitoid *Tetrastichus planipennisi*, became widely established and increased in rates of parasitism. To assess the potential for ash recovery in these stands, we also quantified the abundance and crown condition of the ash saplings and surviving ash trees at the study sites. We found that *T. planipennisi* was the dominant biotic mortality factor in saplings, killing 36–85% of the late instar borer larvae. Neither woodpecker predation nor native parasitoids caused more than minor levels (<20%) of borer mortality in saplings. Life table analyses of these data further showed that the net population growth rate of the pest in saplings was near or under replacement levels, and that the introduced biocontrol agent reduced the pest's net population growth rate in saplings at our study sites by over 50%. In addition, stand inventories found that healthy ash saplings (4–16 per 100 m²) and smaller (pole size) trees (2–9 per 100 m²) remained in the six study sites, despite an early high density population of the pest at the sites. These findings indicate that the introduced biocontrol agent *T. planipennisi* is providing significant biocontrol services, enhancing ash survival and promoting recovery of the ash in southern Michigan.

KEY WORDS: Hymenoptera, natural enemies, predation, parasitism, invasive, life table, wood borer.

1. Introduction

North American forests are frequently invaded by non-native insects due to increasing global travel of people and expanding international trade (Liebhold et al., 1995; Brockerhoff et al., 2006; Aukema et al., 2010, 2011). Some of these invasive insects severely damage North American forest ecosystems (Aukema et al., 2010, 2011; Van Driesche and Reardon, 2014). An understanding of the population dynamics of such invasive forest pests is needed when restoration efforts are initiated after the invasion (Lockwood et al., 2007).

The emerald ash borer, *Agrilus planipennis* Fairmaire, a buprestid beetle native to northeastern Asia, was discovered in North America as the cause of widespread ash tree (*Fraxinus* spp.) mortality in southeast Michigan and nearby Ontario in 2002 (Haack et al. 2002; Cappaert et al., 2005; Poland and McCullough, 2006). Since then, this invasive beetle has spread throughout much of the eastern United States and Canada, where it has killed hundreds of millions of ash trees (Emerald Ash Borer Information, 2017). In Michigan and Ohio, near the epicenter of the invasion, researchers found 99% mortality of healthy overstory ash trees within six years of detection of emerald ash borer (Smith 2006; Knight et al., 2013; Klooster et al., 2014). The ecological impacts of such a rapid reduction in ash abundance include changes in forest succession, species composition, and hydrologic processes (Flower et al., 2013; Slesak et al., 2014; Nisbet et al., 2015), losses in biodiversity of ash-dependent species (Gandhi et al., 2014; Wagner and Todd, 2016; Jennings et al., 2016a), and alterations in nutrient and carbon cycles (Ulyshen et al., 2011, 2012; Stephens et al., 2013; Flower et al., 2014).

Despite predictions that *Fraxinus* could be functionally extirpated from North American forests (Herms and McCullough 2014), research shows that tree mortality is moderated by several factors including ash species and genotype (Liu et al., 2003; Rebek et al., 2008; Koch et

al., 2015); ash tree age, vigor, and stand density (Siegert et al., 2010; Kashian and Witter 2011; Mercader et al., 2011; Knight et al., 2013; Kashian 2016); pest population outbreak stages (Burr and McCullough 2014); climatic factors (Wu et al., 2007; Crosthwaite et al., 2011; DeSantis et al., 2013); and natural enemies (Liu et al., 2003, 2007; Lindell et al., 2008; Duan et al., 2010, 2012a, 2013a, 2015; Bauer et al., 2015; Jennings et al., 2016b, 2016c; Murphy 2017). Moreover, a recent five-year study of green ash (*F. pennsylvanica*) regeneration in the aftermath forests of southeast Michigan revealed seed production in small ash trees and basal ash sprouts in 2011 and subsequent seedling recruitment throughout the region (Kashian, 2016). These findings suggest that green ash will likely persist in the presence of emerald ash borer and may remain an important forest species, although its stature and population densities may be greatly diminished on the landscape (Kashian, 2016).

The natural enemy release hypothesis states that some non-native species achieve pest status because they are accidentally separated from their specialized natural enemies when they arrive to new locations and local species are unable to suppress them (e.g., Keane and Crawley, 2002; Mitchell and Power, 2004; Murphy et al., 2014). Although this hypothesis has been criticized for lack of direct experimental evidence in some cases (e.g., Berdegue et al., 1996; Colautti et al., 2004), many dramatic successes in biological control have resulted from the re-association of such invasive pests with their co-evolved natural enemies from the pests' native ranges (e.g., Embree, 1966; see case reviews in Clausen, 1978; van den Bosch, et al., 1982; Van Driesche et al., 2010; Van Driesche and Reardon, 2014).

Biological control of emerald ash borer was initiated by the United States Department of Agriculture (USDA) shortly after the beetle was detected in North America (Bauer et al., 2008, 2015). Following regulatory review and approval, USDA issued permits in 2007 for the

environmental release of three emerald ash borer parasitoids from northeast China into the United States: the solitary egg parasitoid *Oobius agrili* Zhang & Huang (Hymenoptera: Encyrtidae) and the two gregarious larval parasitoids *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Federal Register, 2007). *Tetrastichus planipennisi* is an endoparasitoid attacking older emerald ash borer larvae (third and fourth instars) (Liu et al., 2003, 2007; Ulyshen et al., 2010), while *S. agrili* is an ectoparasitoid of the same stages (Yang et al., 2005). Another larval ectoparasitoid, *Spathius galinae* Belokobylskij & Strazanac (Braconidae), from the Russian Far East, was approved for release in 2015 in the U.S. (Belokobylskij et al., 2012; Duan et al., 2012b; Federal Register, 2015). Release of *T. planipennisi* and *O. agrili* continues in emerald ash borer-infested regions of the United States (to date, in 26 of the 31 infested states) and Canada (two provinces). These two species have been consistently recovered more than one year after their release and are considered established in northern regions (Duan et al., 2013a; Abell et al., 2014; Bauer et al., 2015; Mapbiocontrol, 2017). Release of *S. agrili*, however, is now limited to regions south of the 40th parallel due to lack of establishment in northern regions, while *S. galinae* has been approved since 2015 for release in regions north of the 40th parallel (USDA-APHIS/ARS/FS, 2016).

Since the start of biocontrol releases in 2007, field studies in different regions of North America have monitored emerald ash borer parasitoid populations for their establishment and prevalence (Duan et al., 2013a, 2014, 2015; Abell et al., 2014; 2016; Bauer et al., 2015; Davidson and Rieske 2016; Parisio et al., 2017; Mapbiocontrol.org 2017). As the pest infestation continues to expand in ash stands in North America, predation of emerald ash borer larvae and pupae by woodpeckers and other bark-foraging birds, and larval parasitism by native

parasitoids via new species associations, are regularly observed both in the invasion's epicenter in Michigan (Lindell et al. 2008; Cappaert and McCullough, 2009; Duan et al., 2010, 2014; Jennings et al., 2016c) and at its expanding edges (e.g., Colorado, Texas, New England, Ontario, and Quebec) (Kula et al., 2010; Duan et al., 2013b; Jennings et al., 2013, 2016b, 2016c; Flower et al., 2014; Roscoe et al., 2016).

A seven-year field study (2008 to 2014) in southern Michigan, following release of the three Chinese parasitoids, showed that parasitism by *T. planipennisi* and *O. agrili* contributed significantly to the reduction of net population growth rate (R_0) of emerald ash borers infesting small to medium ash trees (averaging 8.7–12.1 cm diameter at breast height [DBH]) four years after their initial release (Duan et al., 2013a, 2015; Abell et al., 2014). These biocontrol agents, together with woodpeckers and native parasitoids, primarily *Atanycolus* spp., caused target pest densities in infested ash to decline ~ 90% (Duan et al., 2015). However, studies are lacking on the impact of introduced and native natural enemies on emerald ash borers attacking ash saplings. Saplings are a critical bridge cohort, whose survival is essential as new ash trees develop from understory seedlings, after the loss of overstory trees (Kashian, 2016).

Here we report results of a three-year study (2013 to 2015) on the impact of natural enemies on emerald ash borer population dynamics in saplings (2.5–5.8 cm DBH) at six deciduous forest stands in southern Michigan, where *O. agrili*, *T. planipennisi*, and *S. agrili* were released from 2007 to 2010 and where *O. agrili* and *T. planipennisi* became widely established (Duan et al., 2010, 2013a; Abell et al., 2014). For emerald ash borer life stages found in ash saplings, we constructed lifetables and estimated population growth rates using methods previously applied in larger ash trees at the same sites from 2008 to 2014 (Duan et al., 2014, 2015). Lifetables for the emerald ash borer in saplings allowed estimation of pest population growth rates with and

without larval parasitism and the contribution to pest reduction due to *T. planipennisi*. To provide insights into the potential future survival and recovery of ash in North American forests, we also assessed ash abundance and crown condition for four size-class ash trees at our study sites.

2. Methods

2.1. Site description

Our study on saplings was conducted in six forested sites in three southern Michigan counties: Ingham Co. (three sites), Gratiot Co. (two sites), and Shiawassee Co. (one site), with 10 to 60 km between sites. These sites were primarily early successional, second-growth northern deciduous forests dominated by green (*F. pennsylvanica* Marsh) and white ash (*F. americana* L.). Less abundant trees species in these forests were black ash (*F. nigra* Marsh), red maple (*Acer rubrum* L.), boxelder (*A. negundo* L.), oaks (*Quercus* spp.), black cherry (*Prunus serotina* Ehrh.), aspen (*Populus tremuloides* Michx), eastern cottonwood (*Populus deltoides* Bartr. ex Marsh), black walnut (*Juglans nigra* L.), American basswood (*Tilia americana* L.), and some pine (*Pinus*) species. The location of these sites is described in Duan et al. (2013a).

2.2. Biological control agents released

Each forest site was divided into two plots (each 10 – 20 ha and separated by 1– 6 km), which were randomly designated as either parasitoid-release or non-release control at each

location. From 2007 to 2010, *O. agrili*, *S. agrili*, and *T. planipennisi* were introduced into each release plot. Detailed information on release procedure, timing, frequency, and number of adult wasps released for each species can be found in Duan et al. (2015).

2.3. Sampling procedures for the pest and its natural enemies

We haphazardly selected 10 ash saplings (2.5–5.8 cm DBH) at each of the six release and six control plots at various points from January through April in 2014, 2015, and 2016 (120 saplings/year). Saplings were measured for DBH, felled, labeled in the field, and then returned to the laboratory, stored in buckets of water in a walk-in cold room (4°C) until each was carefully debarked on a shaving bench (Country Workshops, Marshall, NC) with an Austrian drawknife (Lee Valley Tools Ltd., Ogdensburg, NY) for detection of emerald ash borer feeding galleries and recovery of live stages of the pest and associated parasitoids. The observed insects hatched from eggs laid the previous year. In addition to measuring larval parasitism rates in the saplings, we also determined mortality rates associated with other factors, including avian predation (primarily from woodpeckers) and undermined biotic factors such as putative host tree resistance, intraspecific competition, and/or disease.

Procedures for determining the pest's larval instar and the species of associated parasitoids or recognition of other mortality factors upon debarking of the saplings are described in Duan et al. (2015). Briefly, each emerald ash borer gallery or pupation chamber (formed by mature 4th-instar larvae) was examined upon removal of both outer and inner bark tissues on the stems of saplings. For all current galleries, emerald ash borer larval stages were classified as small (1st to 2nd instars, with gallery widths ≤ 2 mm) or large (3rd to 4th instars, including J-shaped, mature

larvae, with gallery widths >2 mm). Each larva was assigned to one of five categories, as described in Duan et al. (2015): (1) insects that had completed their development, evidenced as D-shaped adult emergence holes, (2) live larvae, (3) larvae or pupae removed by avian predators as evidenced by excavation above empty pest galleries or pupation chambers, (4) cadavers of larvae or pupae dead due to undetermined factors (e.g., host tree defenses, pathogens, intraspecific larval competition, or weather), and (5) parasitized larvae, as indicated by the presence of parasitoid eggs, larvae, pupae, cocoons, pharate adults, meconium, or exit holes associated with live or dead host galleries.

Because the signs and symptoms of parasitism may not be always visible externally, each live emerald ash borer larva that did not show any obvious signs of parasitism was dissected under a dissecting stereomicroscope to look for immature parasitoid stages or their remains. Parasitoids could be identified to species in the case of the gregarious endoparasitic biocontrol agent *T. planipennisi* or to genus for the dominant native solitary ectoparasitoids, *Atanycolus* spp., according to procedures described in Duan et al. (2013a).

2.4. Ash abundance and health conditions

In the summer (mid-July to early August) of 2015, eight belt transects (four in each biocontrol-release plot and four in each non-release control plot, at each of the six sites) were haphazardly established inside study plots using Hip Chain Distance Measurers (Forestry Supplier Inc., Jackson, Mississippi). Each transect was 50-m long and 2-m wide (100 m² of area), with transects being at the minimum of 10 m and the maximum of 500 m from each other within each study plot. All ash (saplings or trees with DBH >1 cm) inside each transect area were

recorded and examined for any signs of EAB infestation (including D-shaped exit holes, epicormic growth, and signs of current-year woodpecker feeding). The crown condition of each sapling and tree was assessed using the crown decline and dieback scale of 1 to 5 developed by Smith (2006) and modified with 0.5 increments. Briefly, a score of 1.0 represented an ash with a healthy crown, and scores that ranged from 1.5 to 4.5 indicated increasing crown decline, with the highest score of 5.0 representing a dead crown.

2.5 Data analysis

A mixed-effects linear model for Analysis of Variance (ANOVA), as described in Duan et al. (2015), was used to evaluate differences in the resource-adjusted pest density (number of live emerald ash borer larvae or pupae per m² of phloem area of sampled saplings) between parasitoid-release and non-release control plots. The total phloem area (y) of each sapling was estimated using a second-order polynomial model ($y = 0.024x^2 - 0.307x + 2.63$) as a function of sampling DBH (x) (McCullough and Siegert, 2007). Log likelihood Chi-square tests (based on a nominal logistic regression model of binomial distribution data) were used to evaluate differences in larval mortality rates caused by different parasitoids, avian predators as a group, and undetermined factors. Mortality rates caused by larval parasitoids were calculated as marginal attack rates by excluding from the denominator any pest larvae killed by avian predators or undetermined factors. This calculation of marginal attack rate was based on the assumption that avian predators and agents in the “undetermined factor” category acted on pest larvae contemporaneously with the larval parasitoids and showed no discrimination between healthy and parasitized emerald ash borer larvae (Elkinton et al., 1992; Duan et al., 2015). However, mortality rates from avian predators and undetermined factors were calculated as the

proportion of individuals dying from each of these causes relative to the total number of individuals (dead and live) in all emerald ash borer life stages because these biotic factors occurred at all pest stages, regardless of their condition or previous attack by other factors. All statistical analyses were carried out with JMP Pro 12.01 (SAS Institute 2014), and the outputs, along with statistical program scripts, are presented in Appendix A (Supplementary Information).

Life table construction procedures as described by Duan et al. (2015) were used to construct life tables for pest populations. These tables were based on the observed number of live or dead pest larvae or pupae found each year at the site, by pooling data from the release and control plots at each site. However, unlike in Duan et al. (2015), no additional overwintering mortality was added to calculate the net population growth rate (R_0) in the present study because saplings were sampled from winter through early spring, not in the fall as in Duan et al. (2015). We estimated the number of emerald ash borer eggs for the beginning of the following (F_1) generation from the number of surviving immature stages (3rd and 4th instars), applying a sex ratio of 0.5 to estimate the number of females, and an average of 30 viable eggs per gravid female, as per Rutledge and Keena (2012). The mixed-effects ANOVA model was used to detect the statistical significance of the effect of parasitism, by either *T. planipennisi* alone or in combination with the native parasitoids (*Atanycolus* spp.) on pest population growth rates.

3. Results

3.1 Emerald ash borer infestation rates and resource-adjusted densities

Throughout the three-year study, 20–35% of sampled saplings had signs of current emerald ash borer infestation in both the biocontrol release and non-release control plots at the six study sites (Fig. 1A). When adjusted to the area of ash phloem (per m²), the mean number of live pest larvae (all instars) in the infested saplings was 2–7 per m² in both release and control plots during the study period (Fig. 1B). There was no significant difference in the pest infestation rate between saplings in the release and control plots (logistic regression model: log likelihood $\chi^2 = 0.5857$, $P = 0.7641$), nor were there any significant differences in the mean pest density between the release and control plots (mixed linear ANOVA, $F_{1,73} = 0.3792$; $P = 0.5399$). However, logistic regression analysis revealed significant differences in sapling infestation rates by the pest among different sampling years (log likelihood $\chi^2 = 9.73$, $P = 0.0452$) as well as among different study sites (log likelihood $\chi^2 = 25.83$; $P = 0.0040$).

3.2 Mortality of emerald ash borer larvae from different groups of natural enemies

Two parasitoid groups were observed attacking late-instar pest larvae (3rd and 4th instar) infesting saplings in both release and non-release plots at the six study sites throughout the three-year study period: the introduced biological control agent *T. planipennisi* and native species of *Atanycolus*. *Tetrastichus planipennisi* was the dominant parasitoid (>92% of all cases of larval parasitism), attacking 36–85% of late-instar larvae (Fig. 2A). In contrast, parasitism rate by *Atanycolus* spp. was 1–20% (Fig. 2B) during the study period. There were no significant differences in *T. planipennisi* parasitism between release and control plots (log likelihood $\chi^2 = 3.28$, $P = 0.0694$), nor in parasitism rates by *Atanycolus* spp. between release and control plots (log likelihood $\chi^2 < 0.0001$; $P = 0.9984$). There were no significant interaction effects on

parasitism rates by either *T. planipennisi* or *Atanycolus* spp. between biocontrol treatments (release vs control) and the sampling year (all $P > 0.10$).

Avian predation of immature pest life stages was $<10\%$ throughout the study period in both release and control plots (Fig. 3A). Larval mortality of pests from undetermined factors (e.g., putative plant resistance, pathogens, intraspecific larval competition, and weather) was 2 - 23% (Fig. 3B) in both release and control plots. There were no significant differences in the rates of avian predation between the release and control plots, nor were there any significant interactions between biocontrol treatments and sampling year (all $P > 0.10$). However, the pest mortality rate caused by the undetermined factors was significantly higher in release plots than in control plots in 2014 (log likelihood $\chi^2 = 7.61$; $P = 0.0058$).

3.3. Effect of introduced biocontrol agents on pest net population growth rate

A representative life table, based on the immature stages of emerald ash borer at Rose Lake State Wildlife Area (combining the release and control plot data) for 2015, shows rates of apparent (stage-specific) mortality and associated mortality factors, real mortality, and estimates of pest net population growth rate (R_0) (Table 1). The impact of the biocontrol agent *T. planipennisi* on target pest population growth was assessed by the change in the R_0 value when mortality from *T. planipennisi* was removed from the life table, with the assumption that all subsequent mortality factors would kill the same percentage of the pest (i.e., none were density dependent). When parasitism by *T. planipennisi* was removed from this life table, R_0 values doubled, increasing from 2.2 to 4.4 (Table 1). This change indicates that larval parasitism by *T. planipennisi* reduced the pest population growth at this site by 50%.

Data from life tables constructed for one complete generation in each year at each of the six study sites (pooling data from parasitoid-release and control plots) showed that on average R_0 values were near one, the threshold of replacement (Fig. 4). When parasitism by *T. planipennisi* was removed from the life table, R_0 values for the pest populations increased over 100% in each of the three study years (Fig. 4 – green dotted line), indicating the importance of this biocontrol agent in suppressing population growth of emerald ash borers in ash saplings. Further life table analyses showed that the native *Atanycolus* parasitoids had only a minor effect on pest R_0 values, especially in 2014 and 2015 (Fig. 5 – red dotted line). ANOVA (Appendix A) revealed significant effects of parasitism by *T. planipennisi* (regardless of the presence of the native parasitoids) on population growth rates of emerald ash borers in all three years of the study (mixed linear model, $F_{2,40} = 4.5$; $P = 0.0172$).

3.4. Survival of ash saplings and trees in the study sites

There were no significant differences in either average ash tree size (DBH) or density (number of trees and/or saplings per 100 m² transect) between release and control plots at the six study sites. Pooled data from release and control plots showed that healthy ash saplings and smaller trees (crown classes of 1 to 2) remained abundant in the six study sites. The average density of healthy ash saplings (1–5.8 cm DBH) and small ash trees (5.1–10.1 cm DBH) across different study sites was 4–16 and 2–9 per 100 m² (= 0.01 ha) transect, respectively (Fig. 5A). However, the density of larger healthy ash trees (DBH >20.1 cm) was relatively low (<1 tree per 100 m² transect). The density of declining ash trees (crown classes 2.5 to 3.5), as well as dying or dead trees (crown classes 4 to 5), was low and similar across all sites (Fig. 5B and 5C).

Data pooled from all sites showed that the proportion of healthy ash saplings or trees (with crown classes of 1 to 2) decreased with DBH category (or tree age), from 80% for the saplings (1–5 cm DBH) to < 25% for the large trees (>20.1 cm DBH) (Fig. 6). Nominal logistic regression analysis indicated that the crown class category, as a measure of ash health condition, varied highly significantly among different study sites (Likelihood $\chi^2 = 157.42$; $P < 0.0001$) as well as with the DBH of the trees or saplings (Likelihood $\chi^2 = 132.65$; $P < 0.0001$).

Discussion

A previous seven-year field study conducted at the same locations showed that local, generalist natural enemies such as woodpeckers and native parasitoids provided significant biological control of emerald ash borers infesting small to medium ash trees (averaging 8–12 cm DBH) during the outbreak phase of pest invasion (Duan et al., 2015). However, this same study also showed that the biocontrol service of pest suppression gradually shifted to the introduced specialist parasitoid *T. planipennisi* following the collapse of emerald ash borer populations. Data from the present study showed that the introduced biocontrol agent *T. planipennisi* has also become the dominant biotic factor affecting emerald ash borer larvae attacking ash saplings in aftermath forests affected by the pest invasion. Data on ash densities from 2015, the last year of the present study, also showed that healthy ash saplings and small trees remained in the six study sites. Findings from this study, together with those from the earlier ones on pest populations infesting small to medium size ash trees (Duan et al., 2013a, 2015), demonstrate that the introduced biocontrol agent *T. planipennisi* has firmly established self-sustaining populations in these locations in Michigan and is suppressing pest population densities in these ash-dominant

336 forests. The biocontrol of emerald ash borer by *T. planipennisi* also significantly contributes to
337 the survival of sapling and young ash trees in Michigan and should continue promoting ash
338 recovery.

339 However, previous studies also showed that *T. planipennisi* primarily parasitizes emerald
340 ash borer larvae in smaller trees or branches rather than boles of larger trees (Liu et al. 2007;
341 Duan et al., 2012b; Jennings et al., 2016b), due to bark thickness, which increases with tree size
342 (Abell et al., 2012; Murphy, 2016). The high parasitism rate (36–85%) of late-instar larvae in ash
343 saplings (DBH <5.8 cm) is consistent with this limitation given the better match between the
344 parasitoid's ovipositor length (average 2.0-2.5 mm) and bark thickness in such small trees. Bark
345 thickness is known to prevent parasitoids of this species from attacking host larvae in the lower
346 boles of trees that exceed 12 cm DBH (corresponding to a bark thickness of 3.2 mm) (Abell et
347 al., 2012). To successfully control emerald ash borer in larger ash trees, however, additional
348 efforts are needed to establish a specialized emerald ash borer larval parasitoid with a longer
349 ovipositor, such as the braconid wasp *S. galinae*, which was first released at these study sites in
350 2015. Should *S. galinae* establish, it is likely to be more effective in larger diameter ash trees
351 because of its longer ovipositor (4-6 mm), allowing it to successfully parasitize emerald ash
352 borer larvae in ash trees up to 30-cm DBH (Duan et al. 2012a).

353 In addition, egg parasitoids introduced from emerald ash borer's native range may also
354 help protect all size class ash trees against infestation, as they kill the borers before damage to
355 the tree phloem. However, the current level of egg parasitism by *O. agrili* (<29%, see Abell et
356 al., 2014) is insufficient to protect ash trees by itself. Introduction of other egg parasitoids, such
357 as *O. primorskyensis* Yao & Duan, may enhance egg parasitism (Larson and Duan, 2016).
358 Recovery of native *Fraxinus* spp. in forest stands invaded by emerald ash borer in North

America will require protection and survival of ash trees across their life cycle and across many climate zones.

Avian predation rates of emerald ash borer larvae or pupae in small to large ash trees (primarily by several species of woodpeckers) are high (20–95%) in many regions, including Michigan (Lindell et al., 2008; Flower et al., 2014; Jennings et al., 2013, 2016c). However, low-to-moderate mortality rates (6–28%) of emerald ash borer larvae or pupae by undetermined factors were observed in previous studies conducted at the same locations (Duan et al., 2013a, 2015). In contrast, in the present study there was very little avian predation (<10%) of emerald ash borer larvae and pupae infesting ash saplings, and low-to-moderate level of mortality rates (2 - 23%) from undermined factors. This discrepancy in pest mortality rates caused by avian predators and undetermined factors between the previous and present studies may be due to lower host densities and to difference in the physical and/or chemical properties between ash saplings and mature trees. For example, avian predators (primary woodpeckers) may not prefer foraging on saplings due to its limited support for bird movement while actively feeding. On the other hand, ash saplings may be less resistant to emerald ash borer larvae than are the larger ash trees.

Previous research at the same locations in which we sampled small to medium ash trees, revealed no significant differences in parasitism by *Atanycolus* between release and control plots (Duan et al. 2015). Additionally, parasitism rates by *Atanycolus* spp. in pole-sized trees decreased sharply as emerald ash borer densities collapsed in these study sites (Duan et al., 2015). In the present study of ash saplings, we found very low rates of *Atanycolus* parasitism and no significant differences between release and control plots. Life table analyses from the present study further showed that *Atanycolus* spp. played a non-significant role in reducing

emerald ash borer population growth in saplings. The low level of *Atanycolus* parasitism observed in the present study may be related to the low density of host larvae in saplings rather than interspecific competition with the introduced biocontrol agent *T. planipennisi*. This is because species of *Atanycolus* are generalist parasitoids, which attack many groups of wood-boring beetles occurring at high densities before discovering emerald ash borer as a novel host in North America (Marsh et al., 2009).

Consistent with results from our previous study of emerald ash borer populations in small to medium ash trees (Duan et al., 2015), no significant differences in *T. planipennisi* parasitism rate and pest density were found in ash saplings between the release and control plots. This resulted from the spread of *T. planipennisi* from the release plots to the control plots at each study site. Recent studies have shown that adults of *T. planipennisi* can disperse >1 km within one field season following release (Duan et al., 2012b, 2013, 2015). As demonstrated in this and previous studies (e.g., Van Driesche and Taub, 1983, Jennings et al. 2013; Duan et al. 2014, 2015), construction of life tables of the targeted pest population and subsequent analysis of net population growth rates with and without the biocontrol agent's effect provides a powerful method to quantify the effect of biological control programs on the pest population dynamics.

Unlike the results from our previous study with small to medium ash trees, the mean R_0 value of emerald ash borers in ash saplings was near one in the first two years of the study (2013 and 2014) and slightly below one in the last year of the study (2015). These R_0 values indicate that emerald ash borer populations infesting ash saplings at the current level of densities (2–7 larvae per m² of sampled phloem) are near or below replacement levels and insufficient to kill ash saplings during this study. A recent study in urban forests shows that infested ash trees could recover from a density of 10 emerald ash borer larvae per m² of phloem (MacQuarrie and

Scharbach 2015). Given that the introduced larval parasitoid *T. planipennisi*, as well as the egg parasitoid *O. agrili* (not analyzed in this study, but see Abell et al., 2014) have established stable populations and provide significant pest biocontrol services, we are hopeful that ash saplings in the aftermath forests of southern Michigan can survive sufficiently to successfully reproduce (Kashian and Witter, 2011). Tree survival rates are expected to decline as saplings and small trees mature unless biocontrol protection can be extended to larger trees through the introduction and establishment of new biocontrol agents. To allow for better recovery of North American ash following the invasion of emerald ash borer, we strongly suggest expanded releases of *S. galinae*, which has a considerably longer ovipositor and may complement *T. planipennisi* in regulating borer populations attacking larger size class ash trees. Furthermore, as the emerald ash borer expands its range in North America, we encourage more foreign exploration in Asia for natural enemies adapted to different climate zones.

Acknowledgments

We thank Deborah Miller and Toby Petrice (USDA Forest Service, Northern Research Station), Jonathan Schmude (USDA ARS Beneficial Insects Introduction Research Units), Erin Morris (Michigan State University, Department of Entomology), and many Michigan State University student employees for assisting in laboratory work and field surveys of emerald ash borer larvae and their parasitoids on ash saplings. We are also grateful to Kim Hoelmer (USDA Agricultural Research Service) for helpful comments on an early version of this manuscript, and Richard

Reardon and Steven Katovich (USDA Forest Service, State and Private Forestry) for providing partial financial support for this project.

Appendix A. Supplementary material

These data include scripts and outputs of all statistical analyses using JPM Pro 11.02.

Data Accessibility

Data reported in this article including lifetable parameter estimates are available through the Ag Data Commons (National Agricultural Library, USDA ARS):
<http://dx.doi.org/10.15482/USDA.ADC/1347361> (Reserved)

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Table 1. Life table for emerald ash borer population infesting ash saplings observed in 2015 at Rose Lake Wildlife Area in Shiawassee Co., Michigan, one of the six study sites. Data were pooled at this site from the parasitoid release and non-release control plots for life table construction.

Life stage ^a	l_x	m_x	d_x	d_i	Mortality Factor	$q_x = d_x/l_x$	$q_i = d_i/l_x$	$q = d_x/l_0$
(Egg)	(26)	-	(8)		(<i>Oobius agrili</i> 30%)			(0.300)
L1-L2	18	10	0	0	Parasitism (total)	0.00	0.00	0.00
	-	-	0	0	Undetermined	0	0	0
	-	-	-	0	Avian predation	-	0	-
L3-L4	8	4	4	4	Parasitism (total)	0.5	0	0.1538
	-	-	-	4	<i>T. planipennisi</i>	-	0.5	-
	-	-	-	0	<i>Atanycolus</i> spp.	-	-	-
	-	-	-	0	Undetermined	-	-	-
	-	-	-	0	Avian predation	-	-	-
(Adults)	(4)	(4)	-	(0.2)	(Fungus disease - 5%)	-	-	(0.050)
(Females)	(2)	-	-	-	(Female: male = 1:1)	-	-	-
(F1 eggs)	(60)	-	-	-	(30 fertilized eggs/Female)	-	-	-
R_0	2.2	-	-	-	-	-	-	-
(R_{0-TP})	(4.4)				(<i>T. planipennisi</i> is removed)			

^a Parameters for life stages in parenthesis were calculated based on separate estimates from earlier studies (Abell et al. 2014). Live (m_x) small larvae (L1-L2s) were excluded from parameter estimates because of the observed two-year generation (see Duan et al. 2014). Column headings represent: l_x = number of live EAB entering each stage; m_x = number of live EAB observed at sampling time; d_x = number of dead EAB observed in each stage; q_x = apparent (stage-specific) mortality rate (d_x/l_x); d_i = number of EAB dying by a specific mortality factor; q_i = apparent mortality rate caused by a specific factor (d_i/l_x); q = real mortality (d_x or d_i/l_0), R_0 = net reproductive rate, calculated as the ratio of l_0 (number of eggs estimated to start the life table) divided by l_{F1} (the number of eggs produced by surviving adults). R_{0-TP} = net reproductive rate when the 4 parasitized host larvae by *T. planipennisi* were allowed to survive but subject to the same rates of mortality caused by other biotic factors.

Figure Legends:

Fig. 1. Percentage of ash saplings with current infestations of emerald ash borer larvae (A) and emerald ash borer densities (all instars including emerged adults) per unit area (m^2) of sampled ash phloem (B) in both biocontrol-release and non-release control plots in southern Michigan during the three-year study (2013–2015).

Fig. 2. Percent parasitism of late-instar emerald ash borer larvae by the introduced biocontrol agent *Tetrastichus planipennisi* (A) and the North American native parasitoid *Atanycolus* spp. (B) in both the biocontrol-release and non-release control plots in southern Michigan during the three-year study (2013–2015).

Fig. 3. Percent predation of emerald ash borer larvae and pupae by woodpeckers and other bark-foraging birds (A) and percentage mortality of emerald ash borer larvae by undetermined biotic factors such as putative host tree resistance, disease and/or intraspecific competition (B) in both biocontrol-release and non-release control plots in southern Michigan during the three-year study (2013–2015).

Fig. 4. Net population growth rates (R_0) of emerald ash borer populations infesting ash saplings across different study sites (pooled from both parasitoid release and non-release control plots for each site) in southern Michigan in each of the three years of the study (2013–2015).

Fig. 5. Number of ash trees per 100 m^2 with different crown-condition (CC) classifications at the six study sites in southern Michigan observed in summer 2015. All saplings and trees were classified based on CC scores of 1 to 5 at 10% increments. Healthy Ash Trees are CC range 1 – 2 (A), Declining Ash Trees are CC range 2.5 – 3.5 (B), and dead/dying Ash Trees are CC range 4 – 5 (C). Study sites labeled on the X-axis are: BF = Burchfield Park, CP = Central Park-Nancy

740 Moore Parks (Meridian Township Park), LP = Legg Park-Harris Nature Center (Meridian
741 Township Park), GSW = Gratiot Saginaw State Game Area, MRE = Maple River State Game
742 Area, and RL = Rose Lake State Wildlife Area.

743 **Fig. 6.** Distribution (relative percentage) of different crown-condition (CC) classifications in
744 relation to size (DBH) categories observed in the six study sites located in southern Michigan in
745 summer 2015. Green bars represent healthy trees with CC 1-2, yellow bars declining trees with
746 CC 2.5-3.5, and black bars dead/dying trees with CC 4-5.

749 Fig. 2

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751 Fig. 3

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753 Fig. 4

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759 Fig. 6

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