

Measuring the Impact of Biotic Factors on Populations of Immature Emerald Ash Borers (Coleoptera: Buprestidae)

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Environ. Entomol. 39(5): 1513–1522 (2010); DOI: 10.1603/EN10023

ABSTRACT Cohorts of emerald ash borer larvae, *Agrilus planipennis* Fairmaire, were experimentally established in July of 2008 on healthy green ash (*Fraxinus pennsylvanica*) trees in two wooded plots at each of three sites near Lansing, MI, by caging gravid emerald ash borer females or placing laboratory-reared eggs on trunks (0.5–2 m above the ground) of selected trees. One plot at each site was randomly chosen for release of two introduced larval parasitoids, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae), whereas the other served as the control. Stage-specific mortality factors and rates were measured for all experimentally established cohorts and for associated wild (i.e., naturally occurring) emerald ash borer immature stages via destructive sampling of 2.5 m (above the ground) trunk sections of cohort-bearing trees in the spring and fall of 2009. Host tree defense was the most important mortality factor, causing 32.0 to 41.1% mortality in the experimental cohorts and 17.5 to 21.5% in wild emerald ash borer stages by spring 2009, and 16.1 to 29% for the remaining experimental cohorts, and 9.9 to 11.8% for wild immature emerald ash borer stages by fall 2009. Woodpecker predation was the second most important factor, inflicting no mortality in the experimental cohorts but causing 5.0 to 5.6% mortality to associated wild emerald ash borer stages by spring 2009 and 9.2 to 12.8% and 3.2 to 17.7%, respectively, for experimental cohorts and wild emerald ash borer stages by fall 2009. Mortality from disease in both the experimental and wild cohorts was low (<3%) in both the spring and fall sample periods. In the fall 2009 samples, ≈1.5% of experimental cohorts and 0.8% of the wild emerald ash borer stages were parasitized by *T. planipennisi*. While there were no significant differences in mortality rates because of parasitism between parasitoid-release and control plots, *T. planipennisi* was detected in each of the three release sites by the end of the study but was not detected in the experimental cohorts or associated wild larvae in any of the three control plots.

KEY WORDS parasitoids, predators, host tree defense, *Tetrastichus planipennisi*, *Spathius agrili*

The invasive emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has killed tens of millions of ash (*Fraxinus* spp.) trees in both managed and natural forests throughout the midwestern and northeastern United States since its discovery in 2002 in Michigan and Ontario, Canada (Haack et al. 2002). Assuming that emerald ash borer disperses and survives throughout the range of ash in North America, it could kill many times more trees in the next 10 yr (Kovacs et al. 2010). In the Great Lakes area, emerald ash borer adults normally begin to emerge in late May or early June, and feed on ash foliage for at least 2 wk before mating and oviposition (Cappaert et al.

2005). Mated, gravid females then lay eggs in crevices and under bark flakes on limbs and trunks of ash trees. After eclosion, neonate larvae chew through the bark to reach the phloem, where they feed for several months, sometimes developing through two growing seasons before pupating (Cappaert et al. 2005). After larvae go through three molts, mature fourth instars chew pupation chambers in the outer sapwood or bark. Mature (late fourth instar) larvae then develop a folded “J” appearance and are thus termed “J-larvae” in this study. Usually after a winter chill period, the J-larvae become prepupae, a visibly shorter and rounder stage. Pupation generally occurs in early spring with adults emerging from late spring through early summer. Adults live for 3–6 wk feeding on mature ash leaves and rarely cause any significant damage to the host tree (Bauer et al. 2004). Larvae, in contrast, feed for many months on the phloem and outer sapwood, creating extensive galleries that eventually girdle and kill the tree.

Currently, emerald ash borer has invaded 14 states (Illinois, Indiana, Iowa, Kentucky, Maryland, Michi-

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gan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Virginia, West Virginia, and Wisconsin) (Emerald Ash Borer Information, 2010) and two Canadian provinces (Ontario and Quebec) (Canadian Food Inspection Agency, 2010). Regulatory efforts to contain the pest's spread via early detection, quarantine, and removal of infested ash trees had little effect (Cappaert et al. 2005). Moreover, chemical control cannot be used to protect native ash in forests because of prohibitive cost, general impracticality, and potential harm to the environment (Poland & McCullough 2006). In contrast, biological control using natural enemies (primarily parasitoids) may be a cost-effective and environmentally safe alternative (Bauer et al. 2008).

Current biological control efforts against emerald ash borer in North America are focused on the release of one egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al. 2005) and two larval parasitoids *Tetrastichus planipennisi* Yang (Hymenoptera: Elaphidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae) collected from northern China (Liu et al. 2003; Yang et al. 2005, 2006; Liu et al. 2007; United States Department of Agriculture [USDA] APHIS 2007; Bauer et al. 2008). Parasitoid releases started in Michigan in 2007, Ohio and Indiana in 2008, and in Maryland and Illinois in 2009. Field surveys in Michigan, Pennsylvania, Ohio, and Ontario detected some parasitism of emerald ash borer larvae by native parasitoids (Bauer et al. 2004, Lyons 2008, Cappaert & McCullough 2009, Duan et al. 2009). Predators (predominantly woodpeckers) have been observed to cause high levels of mortality to late instars, prepupae, and/or pupae of emerald ash borer in North America (Cappaert et al. 2005, Lindell et al. 2008). Host-tree resistance and pathogenic microorganisms were shown to influence the survival and reproduction of emerald ash borer in both North America (Bauer et al. 2004, Rebek et al. 2008) and Asia (Liu et al. 2003, 2007; Liu and Bauer 2006). To date, however, integrative studies are lacking on the impact of biotic factors, including the introduced biological control agents, on the population dynamics of emerald ash borer in North America.

In this study, we experimentally established cohorts of emerald ash borer larvae on healthy ash trees and then determined the mortality of those cohorts and that of associated wild (i.e., naturally occurring) emerald ash borer immature stages from different factors, including host tree defense, disease, predation, and parasitism by either introduced or native parasitoids. The current study provides some solutions to challenges for the development of life tables or other quantitative approaches to measuring the impact of natural enemies on emerald ash borer population dynamics.

Materials and Methods

Study Site and Tree Selection. The study was conducted in natural ash forest stands located at three sites in Ingham County near Lansing, MI. Two sites

were ≈ 5 km from each other in Meridian Township (3–5 km east of Lansing). Site one (42° 43'N/84° 25'W) included two contiguous Meridian Township, MI, parks (Central and Nancy Moore Parks). Site two (42°41' N/84°22'W) spanned two other Meridian Township parks (Harris Nature Center and Legg Park). The third site (42°34'N/84°36'W) was located in Holt, MI (≈ 25 km south of Lansing), in the William M. Burchfield Park, ≈ 32 km away from the Meridian Township study sites. At each site, two wooded plots, separated from each other by 0.5–0.8 km, were selected and randomly assigned as either the parasitoid release treatment or the nonrelease control plots. All study plots contained a mixture of mostly deciduous trees, including ash (*Fraxinus pennsylvanica* Marsh, *F. americana* L., and *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 americana* L.), pricklyash (*Xanthoxylum americanum* L.), and a few evergreen trees such as spruce (*Picea* spp.) and pine (*Pinus* spp.). Although there were notable differences in tree species composition, abundance, tree basal area, and tree DBH (diameter at breast height; ≈ 1.5 m above the ground) among the three study sites, these characteristics were similar between plots within sites.

Within each study plot, 10 healthy green ash trees (*F. pennsylvanica*) with no apparent signs or symptoms of emerald ash borer infestation (e.g., bark splits, exit holes, epicormic growth, or woodpecker holes) were selected, marked with colored flagging and aluminum tags, and their DBH determined. There were no significant differences in average DBH of selected ash trees between the parasitoid release plots (mean DBH = 13.6 cm, range 7.5–20.5 cm) and control plots (DBH = 13.2 cm, range 8.5–22.5 cm) ($F = 0.1750$; $df = 1, 58$; $P = 0.6773$). The ash trees selected within each plot were separated by at least 5–10 m.

Creation of Cohorts of Emerald Ash Borer Larvae for Observation. Larval cohorts were established between 29 June and 30 July 2008. Two methods were used to create cohorts of emerald ash borer larvae for observation of survival and mortality rates the following year. The first method involved inserting 3–5 d-old laboratory-reared emerald ash borer eggs under the shallow bark flaps (0.2 cm in depth, 10 mm long \times 5 mm wide) on trunks of selected trees, created by cutting bark with a utility knife. The eggs used were laid by gravid females in the laboratory on small ash sticks (1 cm diameter) wrapped loosely with narrow ribbons that provide sites for emerald ash borer oviposition (L. S. Bauer, unpublished data). Eggs were removed from the sticks by using a utility knife to cut off small pieces of bark flakes (5–7 mm long by 3–5 mm wide) to which eggs were attached. One bark flake, with one or more eggs, was pinned with number one insect pins under each bark flap, leaving enough space between the eggs and the flaps to avoid damaging the eggs. All locations where eggs had been placed were then labeled by writing numbers on the trunk with

weather-resistant ink. Five of the 10 egg exposure sites were low on the trunk (0.5–1 m above ground), while the remaining five exposure sites were 1.5–2.0 m above ground. The field-deployed laboratory eggs were left in place to allow newly hatched emerald ash borer larvae to bore into the phloem and become established. Fates of these larvae were determined through direct observation the following year.

The second method of creating larval cohorts involved caging gravid emerald ash borer females and males to trunks of the same ash trees used for the first method of cohort establishment. Caged females oviposited into natural bark crevices or artificially created bark slits on the trunk. Adult emerald ash borer used in this experiment were collected from heavily infested urban ash trees from mid June to mid July in 2008 in East Lansing, MI. They were held with fresh green ash foliage in ventilated plastic 200-ml cups in the laboratory for ≈ 1 wk before use to ensure that adults had fed and matured eggs. Cages consisted of a ventilated rectangular plastic container (10 cm long \times 7 cm wide \times 4 cm deep) fastened onto the trunk with rubber bands, with the cage opening facing the trunk. Weather stripping (1.5 cm wide \times 0.5 cm thick) was used to fill any gaps between the edges of the open face of the container and the trunk surface. Cages were placed at heights ranging from 0.5 to 2.0 m above ground, with a total of four cages per tree. We placed one male and one female adult emerald ash borer in each cage and provided them with a bouquet of foliage secured in a water-filled vial. In the bark covered by each cage, 5–10 slits in the bark were made to provide sites attractive for oviposition by emerald ash borer females. Emerald ash borer adults in cages were removed after 7 d and caging sites were outlined with weather-resistant liquid Whiteout paint for future reference. At two sites, 10 trees were inoculated in this manner (40 cages per plot). However, at site one (Central Park and Nancy Moore), sufficient wild-collected emerald ash borer beetles were unavailable and only one or two trees per plot were inoculated with emerald ash borer adults (4–8 cages per plot). Approximately 4 wk after the cages were removed, the previously caged areas on each tree were carefully examined by removing any loose bark and counting the numbers of emerald ash borer eggs. At that point in time, all eggs laid by emerald ash borer adults within cages would have hatched (Cappaert et al. 2005), and could be detected and counted because the durable egg shell remains fixed in place.

Parasitoid Releases. After establishment of the sentinel larval cohorts, adult parasitoids were released in both fall of 2008 and summer of 2009. *T. planipennisi* and *S. agrili* were first released between 13 August and 4 October 2008 on each of the 10 study trees in each of the 3 release plots (10 females plus 5–10 males of *T. planipennisi* and eight females and five males of *S. agrili* per tree in a single release).

Further releases were made in 2009 after one half of the emerald ash borer cohort trees had been destructively sampled in the spring of that year. Because of improved rearing methods for *T. planipennisi*, $\approx 3,000$

females (plus 1,000–2,000 males) were released in each release plot. Releases of this parasitoid were staggered, with some adults released every 2 to 3 wk from early June through August in 2009, placing half of the available parasitoids on the 5 remaining emerald ash borer cohort trees and the remainder on 5 adjacent trees.

The second round of *S. agrili* releases was made 24–31 July, 2009, when each release plot received a single release of 200 females and 100 males. Twenty females were placed on each of the 5 remaining emerald ash borer cohort trees and the remainders were placed on 5 other nearby ash trees.

At each release, snap-capped plastic vials (37 ml) containing adults of *T. planipennisi* or *S. agrili* were first opened against the lower (<0.5 m) trunk of each release tree; parasitoids were coaxed to walk or fly out to land on trunk of release trees by gently taping the outside of vials.

Determining the Fate of Emerald Ash Borer Larval Cohorts. Five emerald ash borer cohort trees in each of the 3 release and 3 control plots were destructively sampled in spring 2009 (26 April to 10 May) to determine the developmental stage and fate of the emerald ash borer larvae in each cohort. The remaining 5 emerald ash borer cohort trees were reserved for sampling in fall 2009 (29 September to 8 October). Beginning at the oviposition locations (where either eggs on bark chips had been placed or where adult emerald ash borer had been caged), we debarked the trunk of each emerald ash borer cohort tree and examined each from the ground up to a height of 2.5 m to locate and assess the fate of members of the experimentally established emerald ash borer larval cohort. In addition, we recorded the status (life stage and observable mortality) of all wild emerald ash borer immature stages (larvae, prepupae, pupae) in that sample zone. Larvae in the experimental cohorts were distinguished from wild emerald ash borer stages based on the point of origin of their galleries. However, in $\approx 10\%$ of cases, the identity of some larvae arising from eggs in the experimental cohort was obscured by gallery overlap and these larvae were excluded from experimental cohorts. The life stages of all immature emerald ash borer found were recorded. Larvae were determined to instar based on body size and head capsule width as described in Cappaert et al. (2005) or, when larvae were missing (as from woodpecker predation), an estimate was made based on the width of the gallery (<2 mm wide for first to second instars, 2–3 mm wide for third instars, and >3–4 mm for fourth instars). The fate of each emerald ash borer immature stage was assigned to one of the following 6 categories: (1) complete development (adult emergence hole visible), (2) living emerald ash borer stage, (3) killed by the tree defense (encapsulated by callous tissue), (4) died of disease (cadaver decomposed, often covered with fungal mycelia), (5) preyed upon (partially consumed or missing, with bark and sapwood destruction caused by woodpecker feeding) (Lindell et al. 2008), or (6) parasitized. Deaths categorized as a result of disease were not confirmed with pathogen isolation

Table 1. Comparison of the establishment of emerald ash borer larval cohorts by caging gravid emerald ash borer females vs hand placing laboratory-reared eggs on ash trunks (adults and eggs were deployed July 2008)

Cohort observation time	Cohort creation method	Number of trees used for cohort creation	Number of eggs deployed	No. EAB larvae observed	% (\pm SE) establishment (per tree) ^a
Spring 2009	Caging females	21	84	71	71.2 (\pm 11.8)a
	Placing eggs	30	338	84	26.2 (\pm 3.1)b
Fall 2009	Caging females	22	104	67	73.2 (\pm 7.7)a
	Placing eggs	27	258	37	14.31 (\pm 2.8)b

^a Numbers followed by different letters for each cohort observation time within a season were significantly different according to likelihood χ^2 tests ($\alpha \leq 0.05$).

and so this category might also include insects that died from physiological causes with subsequent growth of decay organisms. Because parasitism was not always evident in the field, all live emerald ash borer larvae were removed from their feeding galleries or pupal chambers using soft forceps, placed into culture plates, and incubated in the laboratory for a maximum of 12 wk to observe developing parasitoids. Parasitoid adults that emerged during laboratory incubation were identified to species (*T. planipennisi*, *Balcha indica* Mani & Kaul) or genus (*Atanycolus* spp.). Emerald ash borer larvae that died during tree dissection or during laboratory incubation were dissected under the stereomicroscope to determine the presence of parasitoid remains, which were determined to species for the two endoparasitoids *T. planipennisi* (gregarious) and *Phasgonophora sulcata* Westwood (solitary), and to genus for the ectoparasitoid, *Spathius* spp. (gregarious) based on the characteristics of the parasitoid larvae, cocoons, or both.

Data Analysis. Likelihood Ratio χ^2 tests were used to compare the success rates of the two cohort creation methods (hand-placement of eggs versus oviposition by caged emerald ash borer adults) as well as to compare development stage distributions of the experimental cohorts versus the associated wild larvae. Densities of wild emerald ash borer immature stage (larvae, J-larvae, prepupae, and pupae) for each tree were calculated as numbers of all wild emerald ash borer immature stages observed per centimeter squared of sampled phloem area, and then square root transformed for two-way analysis of variance (ANOVA) with parasitoid release (versus nonrelease) and study site as two main (effect) factors. Least square mean difference (least significant difference [LSD]) student's *t*-tests were used to separate differences in emerald ash borer densities among different study sites. Percent mortalities were calculated for each biotic factor for both the larvae in the experimental cohorts and the wild larvae from each ash tree. Percentages were then transformed with arcsine square root (x) function for two-way ANOVA with parasitoid release (versus nonrelease) and study site as the two main (effect) factors to compare differences in each category of mortality rates between the parasitoid release and control plots. The inclusion of study sites in the ANOVA model accounted for site-specific effects on each categories of emerald ash borer mortality. The relationships between percentage mortality (dependent variable) caused by the

major mortality factors (host tree defense mechanism and woodpecker predation) and emerald ash borer density (independent viable, i.e., total number of all alive and dead emerald ash borer larvae, prepupae, pupae, and adult exits per meter squared of phloem) were analyzed using liner regression models. All statistical analyses were performed using JMP 8.0.1 (SAS Institute Inc. 2008).

Results

Relative Efficacy of Cohort-Creation Techniques. The establishment rate of larvae from cohorts of eggs laid by caged emerald ash borer females was higher than that from laboratory-produced eggs placed on tree trunks by hand (Table 1). In spring 2009, the mean (\pm SE) larval establishment rate from caged emerald ash borer adults (71.2% \pm 11.8) was significantly higher than from hand-placed eggs (26.2% \pm 3.1) (Likelihood Ratio $\chi^2 = 103.508$; *df* = 1; *P* < 0.0001). Similarly, in the fall of 2009, the mean establishment rate from caged emerald ash borer adults (73.2% \pm 7.7) was significantly higher than from hand-placed eggs (14.3% \pm 2.8) (Likelihood Ratio $\chi^2 = 86.662$; *df* = 1; *P* < 0.0001).

Densities of Wild Emerald Ash Borer Immature Stage in Sampled Portion of Experimental Cohort Trees. In addition to the larvae of the experimentally established cohorts, the sampled zone (0–2.5 m) of the cohort trees contained many wild emerald ash borer immature stages (larvae, J-larvae, prepupae, pupae), in both the spring (23.6 \pm 7.9/m² of phloem) and fall (76.3 \pm 14.8/m² of phloem) sampling periods in 2009. There were, however, no significant differences in the mean densities of wild emerald ash borer immature stages in cohort trees between the release and control plots in either spring (*F* = 0.004; *df* = 1, 25; *P* = 0.9477) or fall (*F* = 0.0651; *df* = 1, 20; *P* = 0.8012). Among sites, there were no significant differences in numbers of wild emerald ash borer immature stages in spring 2009 (*F* = 0.0247; *df* = 2, 25; *P* = 0.9758), but the wild emerald ash borer density did vary significantly among sites in the fall (*F* = 6.1316; *df* = 2, 20; *P* = 0.0084). Densities of immature emerald ash borer stages at site three (William M. Burchfield Park) were significantly higher (range, 126 \pm 37.8–135 \pm 41.5 per m² of phloem) than densities at sites one and two (LSD Student's *t*-tests, *P* < 0.05), which ranged from 33 \pm 29.2–70 \pm 29.3 and did not differ significantly from each other (LSD Student's *t*-tests, *P* > 0.05).

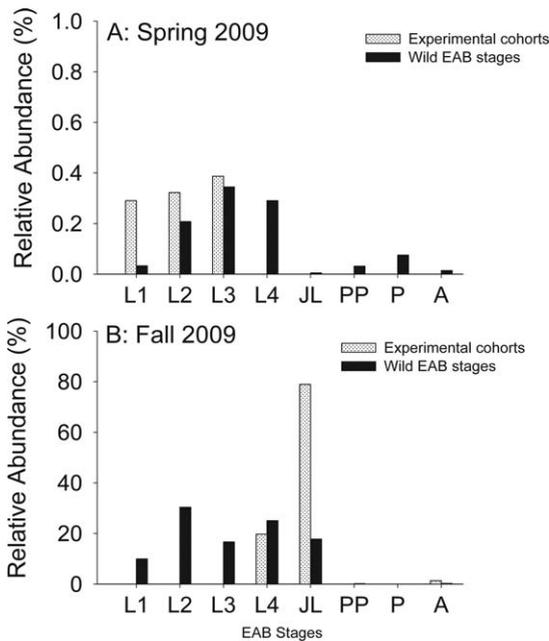


Fig. 1. Relative abundance of immature stages in experimentally established cohorts or among wild emerald ash borer (EAB) immature stages in the sampled zone of emerald ash borer cohort trees, as observed in the spring (A) and fall (B) of 2009. Emerald ash borer life stages: L1-L4 = larval instars first - fourth; JL = J-larva (mature fourth instar with folded "J" appearance), PP = prepupa (short, round appearance), P = pupa, A = emerging adults. For the experimentally established cohorts, ovipositing adults, and eggs were deployed July 2008.

Relative Abundance of Emerald Ash Borer Life Stages in Experimental Cohorts and Wild Emerald Ash Borer Immature Stages in Sampled Cohort Trees. By the spring 2009 sampling period, none of the emerald ash borer larvae in our experimental cohorts (established in July 2008) had yet advanced beyond the third instar (L3) (Fig. 1A). In contrast, ~40% of the wild emerald ash borer immature stages found in the sampled zone of cohort trees were fourth instars (L4's) or older. By the fall 2009 sampling period, all the live larvae in the experimentally established cohort were either L4's or J-larvae (JL). In contrast, the wild emerald ash borer stages in the cohort trees in the fall of 2009 ranged from early instars (L1's to L3's) (56.6%) to L4's and JL (44.4%) (Fig. 1B). The beetle life stage distributions (relative abundance) between the experimentally established cohorts and the wild emerald ash borer immature stages in cohort trees were significant in both spring (Likelihood ratio $\chi^2 = 196.209$; $df = 1$; $P < 0.0001$) and fall (Likelihood ratio $\chi^2 = 166.632$; $df = 1$; $P < 0.0001$). Data from observations of the experimentally established cohorts and the associated wild emerald ash borer immature stages strongly indicated that under the conditions at the study sites, emerald ash borer required more than 1 yr to complete a generation. Late instars (L4's, JL, prepupae) of the previous year's generation of the wild population

clearly overlapped with early instars (L1's to L3's) from the current year's generation in spring.

Stage-Specific Survivorship and Mortality of Experimental Cohorts and Associated Wild Emerald Ash Borer Immature Stages. The major source of mortality in spring 2009 samples was death of larvae from host tree defense (i.e., larvae encapsulated by callous tissue). Young larvae were most affected by this factor, with 82% of L1's in the experimental cohorts being killed and 52% of wild larvae. For L2's, 16% of experimental cohorts and 11% of wild larvae were killed by tree resistance (Fig. 2A and B). Averaged over all larval instars in samples, 29% of larvae (only L1's and L3's were present) in the experimentally established cohorts and 5% of the associated wild emerald ash borer life stages (which included L1's through pupae) were killed by tree resistance. Losses to predation were not found in the experimentally established cohorts in the spring 2009 samples, but were observed among wild emerald ash borer immature stages, with 81 individuals (11% of stages in samples) killed by woodpeckers (Fig. 2B). The exact stages killed by woodpecker predation could not be determined, but based on gallery size and position in the wood, these were most likely older stages (L4, prepupae, or pupae). Disease was not observed to cause any significant level of mortality in either group in the spring samples. Approximately 2% of the 60 L3s in the experimental cohorts were killed by what we assumed were pathogens, as were 3% of the 538 wild larvae present as L2's to L4's. No parasitism was observed in either the experimentally established cohorts or associated wild emerald ash borer immature stages in the spring 2009 samples.

In the fall 2009 samples, host tree defense was again the dominant mortality factor, killing all larvae that had not advanced to L4 (100% of 18 individuals), which was 17% of all larvae in the experimental cohorts (Fig. 2C). In contrast, only small numbers of the associated wild emerald ash borer larvae were killed by host tree resistance (3% of 2038 individuals in stages L1-JL) (Fig. 2D). Woodpecker predation removed 9% of the emerald ash borer stages in the experimental cohorts (nine individuals, Fig. 2C) and 5% of the wild emerald ash borer immature stages (102 individuals, Fig. 2D). Disease caused by unidentified pathogens caused <3% mortality spread across various life stages in both the experimentally established cohorts and the associated wild emerald ash borer immature stages. Parasitism, primarily by the released larval parasitoid, *T. planipennisi*, was detected at a low level (<1.5%), in both the experimentally established cohorts (in JL stage) and the associated wild emerald ash borer immature (L2 to L4) stages. In addition to *T. planipennisi*, two native parasitoids (*Atanycolus* spp. and *P. salcata*) and one inadvertently introduced exotic species (*B. indica*) were observed parasitizing wild emerald ash borer larvae, causing <0.2% parasitism. Two cocoons of a *Spathius* sp. were collected from one emerald ash borer larva in the fall of 2009; however, the identity of this species could not be confirmed because the adults did not emerge.

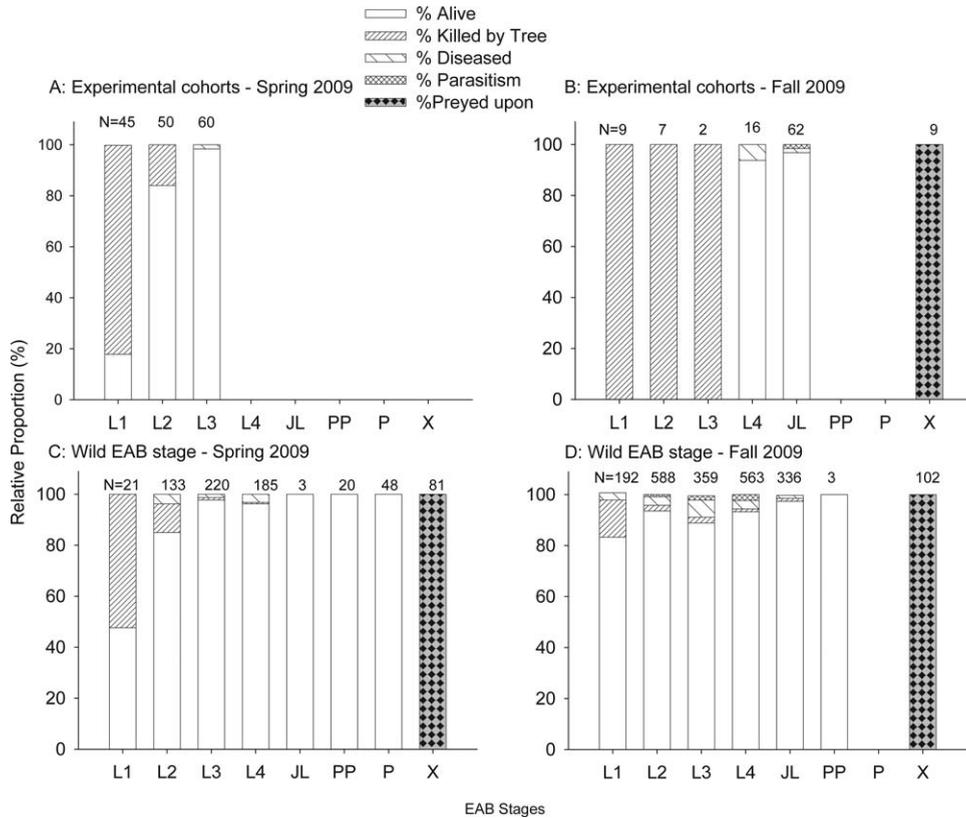


Fig. 2. Relative abundance of various stage-specific mortality factors affecting the experimentally established cohorts and associated wild emerald ash borer (EAB) immature stages in the sampled zone of emerald ash borer cohort trees, as observed in the spring (A and B) and fall (C and D) in 2009. Emerald ash borer life stages: L1-L4 = larval instars 1-4; JL = J-larva (mature fourth instar with folded “J” appearance), PP = prepupa (short, round appearance), P = pupa, X = Unknown instars removed by woodpecker predation. Values above each bar are numbers of each life stage in data set.

Comparison of mortality rates between parasitoid-release and nonrelease plots. For observations in the spring of 2009 (Fig. 3A and B), mean mortality rates (\pm SE) from host tree defense mechanisms were 32.0 (\pm 8.7) at release plots and 41.1% (\pm 9.6) at nonrelease plots for the experimentally established cohorts and 21.5% (\pm 12.1) at release plots and 17.5 (\pm 10.1%) at nonrelease plots for the wild emerald ash borer immature stages in cohort trees. While no woodpecker predation was observed for the experimental cohorts of emerald ash borer larvae in the spring sample, mean predation for the associate wild emerald ash borer stages was 5.6% (\pm 3.0) and 5.0% (\pm 3.1) in the parasitoid release and nonrelease sites, respectively. Mortality caused by unknown diseases (cadaver decomposed and often covered with fungal mycelia) was <2% in both parasitoid release and nonrelease plots. No parasitism was observed in either the parasitoid release or control plots in the spring of 2009. For both experimentally established cohorts and wild cohorts, no significant differences in the mortality rates caused by any of the observed biotic factors were detected between the parasitoid release and nonrelease control plots (ANOVA, all $P_s > 0.05$).

For observations in the fall of 2009, mean mortality rates (\pm SE) inflicted by host tree defense mechanism were 29.2% (\pm 12.7) in release sites and 16.1% (\pm 8.8) in nonrelease control sites for the experimentally established emerald ash borer cohorts and 9.9% (\pm 8.9) in release sites and 11.8% (\pm 3.9) in nonrelease control sites for the associated wild emerald ash borer immature stages. Woodpecker predation resulted in 12.8% (\pm 8.9) and 9.2% (\pm 4.4) mortality for the experimentally established cohorts at release and nonrelease plots, respectively. For wild emerald ash borer immature stages, mortality from woodpecker predation was 17.7% (\pm 8.6) and 3.2% (\pm 2.0) for the parasitoid release and nonrelease plots, respectively. Mortality from disease was <3% in both parasitoid release and nonrelease control sites. No parasitism by the released larval parasitoids (*S. agrili* and *T. planipennisi*) was observed for either the experimentally established or wild cohorts of emerald ash borer larvae in the control plots. However, at parasitoid release plots, a mean of 1.5% (\pm 1.5) parasitism by *T. planipennisi* was observed in the experimentally established cohorts, and 0.8% (\pm 0.5) parasitism was observed for wild emerald ash borer immature stages. Again, for both experimentally

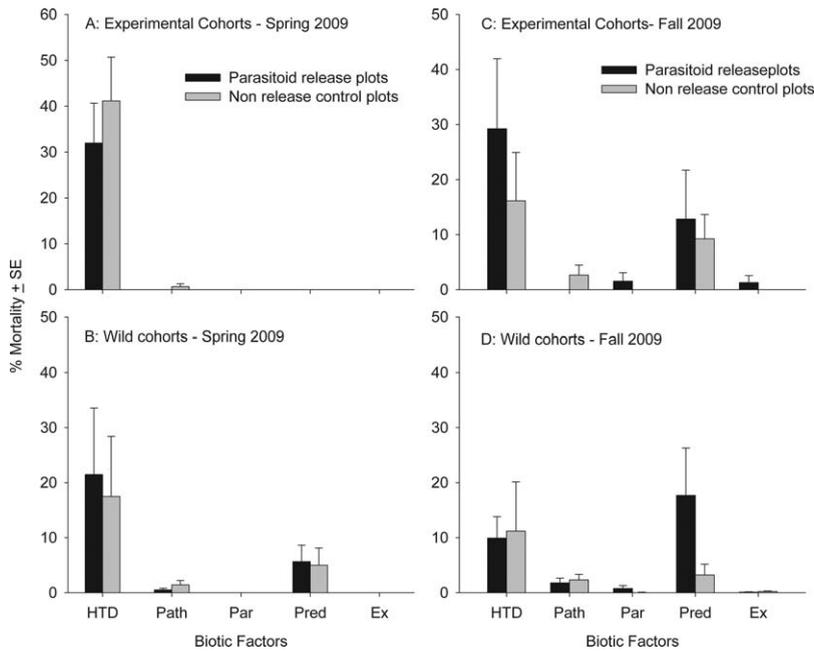


Fig. 3. Mortality rates of experimentally established cohorts and wild emerald ash borer immature stages (wild cohorts) caused by different biotic factors (HTD = host tree defense, Path = disease by pathogens, Par = parasitism (by *T. planipennis* and all other minor parasitoids), Pred = woodpecker predation, and EX = adult exit holes) in parasitoid release and nonrelease control plots in spring (A and B) and fall (C and D) of 2009.

established cohorts and wild cohorts, no significant differences in the mortality rates caused by any of the observed biotic factors were found between the parasitoid release and nonrelease control plots (ANOVA, all $P_s > 0.05$).

Mortality Rates versus Emerald Ash Borer Density.

Regression analyses indicated that mortality rates inflicted by the most common mortality factor, host tree resistance, in both experimental cohorts and associated wild emerald ash borer immature stages were inversely related to emerald ash borer density within individual trees (adjusted for phloem area) both in spring (Fig. 4A and B) and fall (Fig. 4C and D) of 2009, although the relationship was not significant in spring 2009 for wild larvae (LSD tests, all $P > 0.05$). As the emerald ash borer density on the ash trees increased, mortality from host tree defense declined. Additional analysis indicated that there were no significant differences in the mortality rates caused by host tree resistance among the three study sites. Prey density per tree did not appear to significantly affect the rate of woodpecker predation on emerald ash borer immature stages in experimentally established cohorts or on associated wild emerald ash borers in either spring or fall of 2009, whereas marginally significant differences were found among different study sites in woodpecker predation rates on wild emerald ash borer immature stages in emerald ash borer cohort trees in spring ($F = 3.4303$; $df = 2, 16$; $P = 0.0576$) and significant differences were found in fall ($F = 4.6803$; $df = 2, 19$, $P = 0.0223$) of 2009 (data not shown). Mortality rates from disease or parasitism were too low to allow any meaningful analysis of density dependence for either the

experimentally established cohorts or wild emerald ash borer immature stages in emerald ash borer cohort trees in the spring or fall of 2009.

Discussion

Cohorts of emerald ash borer larvae were successfully established by caging gravid emerald ash borer females on trees and by placing laboratory-reared emerald ash borer eggs directly into artificially created bark crevices. However, caging adults resulted in over three times higher rate of larval establishment than direct egg placement. The lower success rate (14–27%) from placement of laboratory-reared eggs into artificial bark crevices may have resulted from damage to eggs during handling or lower rates of success by neonates in tunneling into bark because of the small gap between the eggs and bark. In contrast, cohort establishment via manipulation of emerald ash borer adults placed on tree trunks in cages, resulted in fully natural egg placement and over 70% success in transition from egg to emerald ash borer larvae established in the phloem. Considering the above difference and the great difficulty in finding naturally occurring eggs laid by wild females, caging gravid emerald ash borer adults on tree trunks is the most effective method to establish cohorts of emerald ash borer larvae to estimate emerald ash borer cohort parameters under various ecological conditions.

Previously, some emerald ash borer populations in Michigan were observed to require 2 yr to complete development, especially in newly infested ash trees

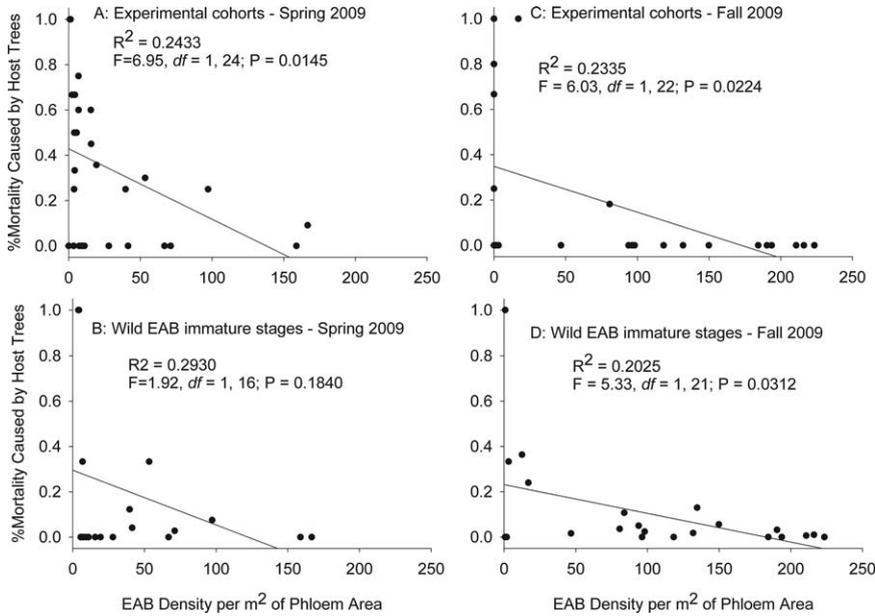


Fig. 4. Relationship between mortality from host tree defense and emerald ash borer (EAB) density (per meter squared of phloem) within individual trees in the spring (A and B) and fall (C and D) of 2009.

(Cappaert et al. 2005, Siegert et al. 2007). In agreement with these observations, we found that artificially established cohorts of emerald ash borer larvae in healthy green ash trees required more than 1 yr to complete their life cycle. Observations of wild emerald ash borer immature stages in our emerald ash borer cohort trees provided further evidence that the emerald ash borer populations we studied in Michigan have overlapping 2-yr long generations in the field, with a mixed population of first-season (first to third instars) and second-season (fourth instars to prepupae) immature stages being present in both spring and fall. 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 establishment and impact of biological control agents.

Resistance of ash trees to emerald ash borer infestation was previously assumed to occur primarily in Asiatic species such as *F. mandshurica* Ruprecht and *F. rhynchophylla* Hance, the species with which emerald ash borer has co-evolved (Liu et al. 2007, Rebek et al. 2008). However, Anulewicz et al. (2008) noted differences in emerald ash borer larval density and development on some North American species of ash trees (e.g., *F. americana* versus *F. pennsylvanica*). Previous studies on other *Agrilus* borers also showed that survival of these borers is highly dependent on host tree condition and any weakening factor such as defoliation, mechanical injury or previous damage by the same or other species of borers increases tree susceptibility to attack and enhances borer survival (e.g., Barter 1957, 1965; Carlson and Knight 1969; Cote and Allen 1980; Haack and Benjamin 1982). However, these earlier studies attributed low *Agrilus* borer infestation rates on healthy host trees to a lack of attraction of host trees to adult beetles or to tree tolerance of

larval feeding damage (also see McCullough et al. 2009 for emerald ash borer). Findings from this study indicate that host tree defense mechanisms are present in North American ash species, which are capable of killing young emerald ash borer larvae at some densities. The lack of increase in emerald ash borer larval mortality from tree resistance in the fall samples was somewhat surprising, but may indicate that host tree defense mechanisms affect primarily the earlier larval stages present in the spring and early summer, when tree growth rate is greatest. We also noted differences in mortality because of host tree defenses between experimentally established cohorts (16–29%) and wild larvae (10–17%). The higher mortality rate in experimentally established cohorts was probably because of experimental procedures, which involved handling and use of laboratory reared eggs and adults.

Ash tree defense may be a combination of chemical and physical mechanisms, although during the course of this study we were only able to observe physical defenses in which emerald ash borer galleries and larvae were surrounded and occasionally absorbed by the formation of callus tissue. Furthermore, rates of mortality from host tree defense were inversely related to emerald ash borer larval density. As the emerald ash borer larval density increases, host tree defense mechanisms become overwhelmed. This phenomenon of overcoming host tree resistance or defense mechanisms through concentrated host attacks is also observed in other *Agrilus* borers [e.g., *A. liragus* (Barter & Brown) in Barter 1965; *A. bilineatus* (Weber) in Cote and Allen 1980] and bark beetles (e.g., *Dendroctonus ponderosae* Hopkins in Raffa and Berryman 1983). Future studies of integrated emerald ash borer management are needed to determine the

threshold of emerald ash borer density that overcomes host tree defense mechanisms, and to what extent host tree defenses would facilitate tree survival if emerald ash borer population densities were reduced by introduced biological control agents.

Several previous studies on emerald ash borer have reported that woodpecker predation is an important mortality factor in some locations in Michigan, killing up to 95% of the large larvae and pupae (Cappaert et al. 2005, Lindell et al. 2008). In contrast, mortality from woodpecker predation observed in this study ranged from 0 to 12.8% for the experimentally established cohorts and from 3.2 to 17.7% for the naturally occurring wild larvae. These lower estimates of woodpecker predation in our study were most likely because of the fact that previous estimates were based on observations of the removal of large emerald ash borer larvae, prepupae, or pupae versus the number of emerald ash borer adult exit holes, which overlooks the presence of live larvae still in the phloem (e.g., Cappaert et al. 2005), whereas in our study all surviving emerald ash borer larvae were included in the calculation of mortality rate. However, woodpecker predation is normally more prevalent in the winter and had our observations been conducted in spring 2010, more cohort or wild emerald ash borer larvae might have been removed by foraging woodpeckers during winter. Lindell et al. (2008) reported that woodpecker predation was positively associated with the emerald ash borer density in a tree. However, positive density-dependence was not evident in this study, possibly because emerald ash borer infestations and rates of woodpecker predation in the sampled ash trees were too low. Consistent with observations by Lindell et al. (2008), woodpecker predation rates appeared to vary significantly among the study locations.

Bauer et al. (2004) isolated five species of pathogens from various immature emerald ash borer stages, with *Beauveria bassiana* (Balsamo) Vuillemin, the most common species, causing <2% mortality of emerald ash borer larvae in Michigan. In this study, a similar low level of mortality from disease was observed. In addition, Bauer et al. (2004) reported that emerald ash borer larvae are parasitized by several endemic and naturally adventive species of hymenopteran parasitoids, including *Atanycolus* spp., *Spathius simillimus* Ashmead, *P. sulcata*, and *B. indica* in Michigan, but only at an extremely low rate (0.05%). Similarly, in our study only one or two individuals of wild emerald ash borer larvae were attacked by these endemic and exotic parasitoid species. A somewhat higher rate of parasitism was observed for the introduced parasitoid *T. planipennisi*, which attacked \approx 1.5% of the emerald ash borer larvae in our parasitoid-release plots. The level of parasitism by *T. planipennisi* seen to date in our plots is still considerably lower than levels (22–40%) reported from China (Liu et al. 2007). This is not surprising considering the short time period (<1 yr) since *T. planipennisi* was first released at these sites (fall 2008). Newly introduced parasitoids normally take several years to a decade to exert significant impact on populations of their hosts.

While the combined impact of these factors on populations of emerald ash borer in this study was low, this merely reflects the fact that there has not yet been time for population increase of the newly established parasitoids. This study provides information on emerald ash borer mortality characteristic of the conditions before the biological control program. It also documents the potential establishment of *T. planipennisi*. Continued monitoring of these sites is planned and will allow us to observe changes in the degree of mortality because of the introduced biological control agents or other factors.

Finally, we point out that patterns of emerald ash borer mortalities observed in this study were from sampling the lower 2.5 m of lightly infested ash tree trunks, and might differ from emerald ash borer mortalities in the upper portion of the trees. Like most other wood-boring *Agrilus* species (e.g., *A. anxius* in Barter 1957, *A. bilineatus* in Haack and Benjamin 1982), emerald ash borer attacks on large ash trees often start in canopy, and move downward in subsequent years (Cappaert 2005). This “downward” infestation pattern is generally attributed to the lower resistance in the tree canopy (e.g., Cote and Allen 1980, Haack and Benjamin 1982) than in the trunk. However, our recent observation of emerald ash borer infestation on small ash trees (DBH range from 6 to 20 cm, similar to those used in this study) indicated that although emerald ash borer attacks might start from the upper trunk in the canopy, emerald ash borer densities per square phloem area were usually several times higher at the lower (3m) trunk (J. J. Duan, unpublished data) than the upper trunks and branches in the canopy. Nevertheless, further studies are needed to determine if the major biotic factors (host plant defense and woodpecker predation) observed in this study have differential impacts on survival of emerald ash borer in different part of the tree, and whether this would affect the efficacy of released biocontrol agents in suppressing emerald ash borer populations at different sections of infested trees.

Acknowledgments

We thank student employees Anthony Paul Capizzo and Jane Slater, and Debbie Miller of the USDA Forest Service Northern Research Station, East Lansing, MI, for assistance in the laboratory and field. We are also grateful to Roger Fuester and Doug Luster (USDA Agricultural Research Service, Beneficial Insects Introduction Research Units) for helpful comments to the earlier version of the manuscript. The project is supported partly by USDA Forest Service State and Private Forestry.

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Received 25 January 2010; accepted 23 June 2010.