

# Establishment and Early Impact of *Spathius galinae* (Hymenoptera: Braconidae) on Emerald Ash Borer (Coleoptera: Buprestidae) in the Northeastern United States

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## Abstract

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, a buprestid beetle native to Asia, has become a serious pest of ash trees (*Fraxinus* spp.) in North America since the early 2000s. Due to the impracticality of applying insecticides in natural forests, biocontrol is the most viable method to manage EAB in natural ecosystems. Here, we report the first evidence for the establishment and impact of *Spathius galinae* Belokobylskij & Strazenac, a larval parasitoid first released in North America in 2016 and 2017 at six mixed-hardwood forest sites, in Connecticut, New York, and Massachusetts. We also report current levels of abundance and parasitism of another introduced larval EAB parasitoid, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), released in 2015 and 2016 in these same sites. *Spathius galinae* was recovered at all release sites in 2018, and its density in sampled trees had increased 1.5- to 20-fold (relative to the first postrelease sample year), reaching a final density of 2.3–14.3 broods/m<sup>2</sup> of phloem area and causing 13.1–49.2% marginal rate of parasitism at four of the six sites. In contrast, *T. planipennisi* was only recovered in 2018 at four of the six release sites, and both its density (0.1–2.3 broods/m<sup>2</sup> of phloem area) and parasitism (0.1–5.6%) were lower than that of *S. galinae* throughout the study at the four sites where recoveries were made. Our data fill a critical gap in the development of a biocontrol-based EAB management plan to protect surviving ash trees capable of reaching maturity and producing replacement trees.

**Key words:** natural enemy introduction, invasive, wood borers, parasitoid release, establishment

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), native to Asia, is a destructive, phloem-feeding pest of ash trees (*Fraxinus* spp.). Since its first detection in southeast Michigan, United States, and Ontario, Canada in 2002, this invasive beetle has seriously degraded ash-dominated forests in 35 U.S. states and four Canadian provinces (Haack et al. 2002, Cappaert et al. 2005, Poland and McCullough 2006, Herms and McCullough 2014, Canadian Food Inspection Agency 2019, Emerald Ash Borer information 2019). Currently, management options for this invasive beetle in North America include trunk injections of systemic pesticides, removal of infested trees, and biocontrol via introduction and establishment of specialized natural enemies from the pest's native range (northeast Asia). Largely because of the cryptic life cycle of

the EAB, lack of efficient detection methods, and impracticability of applying insecticides in natural forests, biological control is the most effective option for use in natural forests (Duan et al. 2018).

The EAB population in North America likely originated in northern China (Bray et al. 2011). Exploration for natural enemies of EAB in its native habitats in China (pre-2007) resulted in the collection of three hymenopteran parasitoids useful as biocontrol agents (Yang et al. 2005, Zhang et al. 2005, Bauer et al. 2007, Liu and Buaer 2007, Liu et al. 2007, Bauer et al. 2008). These agents were the larval parasitoids *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Yang et al. 2006) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al. 2005), and the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera:

Encyrtidae) (Zhang et al. 2005). Following the approval by U.S. federal and state regulatory agencies (Federal Register 2007, Bauer et al. 2008), these species were released in 2007 in small numbers in Michigan, the epicenter of the EAB invasion. After the development of mass-rearing techniques, large numbers of these parasitoids were produced at a specialized production facility in Brighton, Michigan, for more extensive releases in the United States. By the end of the 2018 field season, two or more species of these parasitoids had been released in 26 of the 35 U.S. states (plus Washington, DC) and two of the four Canadian provinces invaded by EAB (Duan et al. 2018, MapBiocontrol 2019, Canadian Food Inspection Agency 2019).

Among the three biocontrol agents released between 2007 and 2012, the eulophid larval parasitoid *T. planipennisi* and the encyrtid egg parasitoid *O. agrili* are considered established in many regions of the United States and have spread naturally to new areas (Duan et al. 2013; Abell et al. 2014; Jennings et al. 2016; 2018; Duan et al. 2018; Jones et al. 2019). Field studies have reported high rates of parasitism (30–85%) by *T. planipennisi* on late instar (third to fourth) EAB larvae in ash saplings (<6 cm in diameter at breast height [DBH]) 5–7 yr after release in Michigan (Duan et al. 2017). In contrast, parasitism by the egg parasitoid *O. agrili* varied from 1 to 32% across different release areas (Abell et al. 2014, Davidson and Rieske 2016, Jennings et al. 2018). However, establishment of the braconid *S. agrili* (primarily released to areas at or below the 40th parallel) occurred only in Maryland (~38th N. L.), where it was recovered 7 yr after release, causing <1% parasitism (J. J. D., unpublished data, also see Hooie et al. 2015 for recovery in other release areas).

Although *T. planipennisi* can protect ash saplings and basal sprouts from EABs in recovering forests postinvasion (Duan et al. 2017, Kashian et al. 2018), this parasitoid becomes less effective as ash trees mature (Abell et al. 2012). This reduction in efficacy occurs because *T. planipennisi*'s short ovipositor (<2.5 mm) limits its ability to reach host larvae beneath bark >3.5 mm thick, which occurs in trees >12 cm DBH (Abell et al. 2012). Although *S. agrili*, which has an ovipositor longer than that of *T. planipennisi*, can attack EAB in larger ash trees (Gould et al. 2011), it did not establish in the northern portions of North America. Recent observations suggest that the life history of *S. agrili*, which was collected in central China, is poorly synchronized with the timing of EAB larvae of suitable size in the northern United States (J. R. G., unpublished data). Therefore, another specialist braconid with a long ovipositor that parasitizes late-instar EAB larvae, *Spathius galinae* Belokobylskij & Strazencac, was collected in the Russian Far East, north of areas where *S. agrili* was collected. *Spathius galinae* was approved for release in the United States in 2015 (Belokobylskij et al. 2012, Duan et al. 2015a, Federal Register 2015). This braconid's ovipositor is slightly longer than that of *S. agrili* and twice that of *T. planipennisi*, allowing it to attack larvae in larger, reproductive-age trees (Murphy et al. 2017). Pre-release climate matching indicated that *S. galinae* from the Russia Far East is well suited to the climate of the north central and northeastern United States, where ash is abundant (USDA APHIS 2015, Duan et al. 2018). In 2016, releases of *S. galinae* were made in Connecticut, Massachusetts, and New York, followed later by releases in the northcentral and Great Lake states (MapBiocontrol 2019). However, little information is available on this parasitoid's ability to overwinter and establish in these regions.

Here, we report establishment and parasitism rates of *S. galinae*, along with those of the other introduced species (*T. planipennisi*), from 2015 to 2018 at six mixed hardwood forest sites with abundant ash in the northeastern United States (three in Connecticut, two in New York, and one in Massachusetts). In addition to recording parasitism by the two introduced larval parasitoids, we

also documented EAB larval density and mortality caused by woodpeckers, undetermined factors (host tree resistance, diseases, and weather), and parasitism by resident (adventive or native North American) parasitoids at each study site. Data from this study fill a critical gap in the development of the biocontrol-based EAB management plan to protect saplings and surviving ash trees capable of reaching maturity. Previous work with *T. planipennisi* has demonstrated its ability to protect small trees (<6 cm DBH), which now experience very low rates of successful EAB attack in our Michigan biocontrol sites (Duan et al. 2017). Also, EAB attack rates on pole size trees (6–12 DBH) in Michigan are now reduced to about 10% of those at the peak of the outbreak in the study area in 2010, due to a variety of factors, including two established Chinese parasitoids (the egg parasitoid *O. agrili* and the larval parasitoid *T. planipennisi*), native parasitoids (especially species of *Atanycolus* braconids), and predation by woodpeckers (Duan et al. 2015b). Here, based on research in forest plots in the northeastern United States, we report that *S. galinae* can establish and attack a large portion of EAB larvae in pole size trees (the size we sampled) and, based on laboratory studies (Murphy et al. 2017), should also be doing the same on larger trees (15–50 cm DBH), which were not sampled directly in our studies due to difficulty of safely cutting trees of this size in the field and time needed for debarking such large trees.

## Materials and Methods

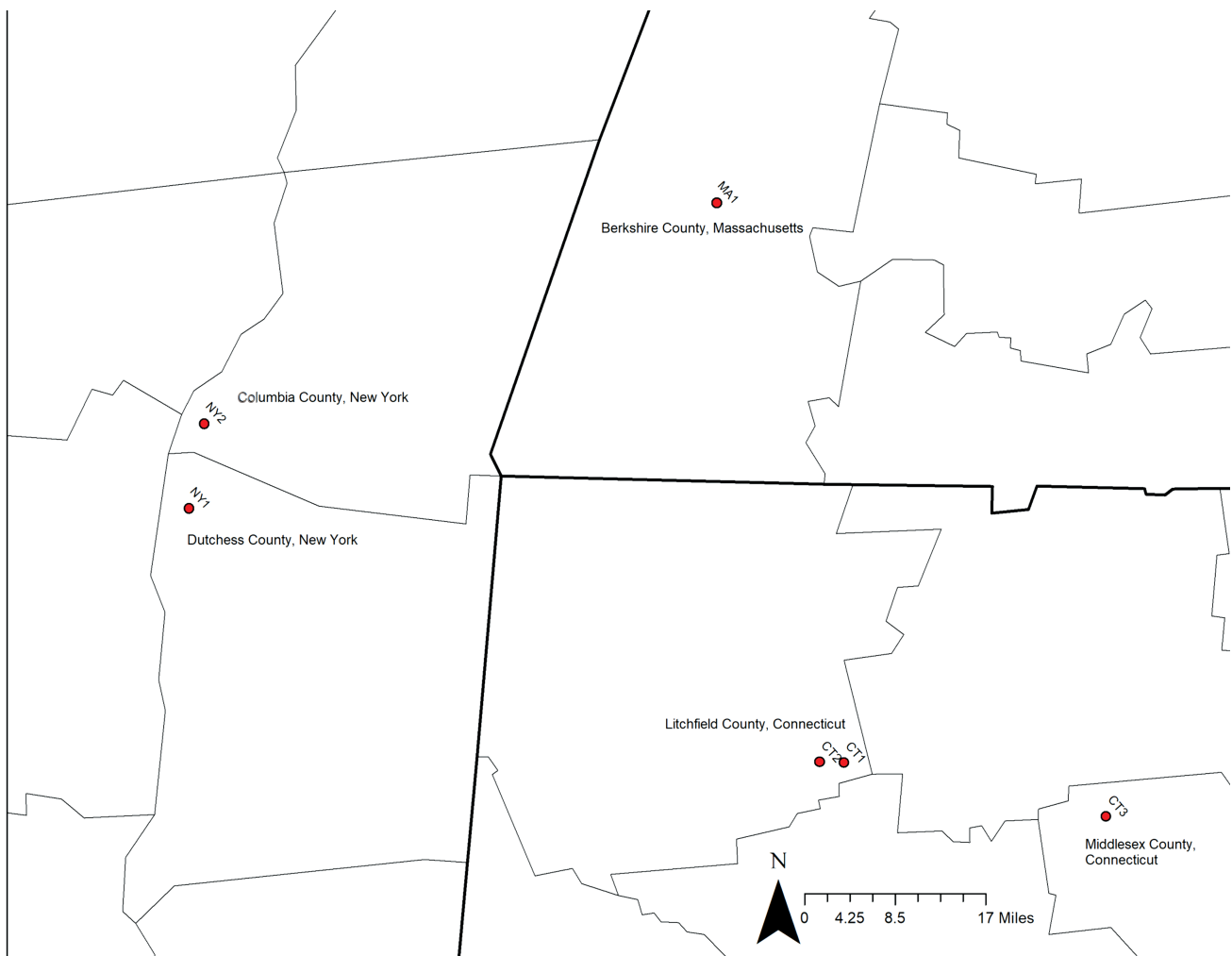
### Study Sites

Our study was conducted in six, mixed hardwood forests (sites) located in three northeastern states: Connecticut, New York, and Massachusetts (Fig. 1). There were three sites in Connecticut: two in adjacent State forest lots (approximately 5–8 ha, N'41.65799, W'73.0182 and N'41.65924, W'73.0214, respectively) in Litchfield County in western Connecticut (hereafter referred to as CT1 and CT2) and one in central Connecticut in the Cromwell Wildlife Management Area (~75 ha, N'42.58519, W'72.6620) in Middlesex County in the flood plain of the Connecticut River (hereafter referred to as CT3). The study site in Massachusetts was a public watershed forest (~22 ha, N'42.41854, W'73.1914) in Berkshire County (MA1). The two study sites in New York consisted of one private conservation forest (~14 ha, N'42.00359, W'73.9087) located in Dutchess County (NY1) and one private, on-farm, woodlot (~3 ha, N'42.11884, W'73.8878) surrounded by other private forest lots (~50 ha) in Columbia County (NY2).

There were notable differences in tree species composition, abundance, tree basal area, and average tree DBH among the six study sites. Major stands of white ash (*Fraxinus americana* L.) along with some green ash (*Fraxinus pennsylvanica* Marshall) dominated CT1 and CT2, whereas green ash was the only ash species at CT3, a flood plain forest dominated by silver maple (*Acer saccharinum* L.). White ash was the dominant tree species in MA1, but both NY sites were dominated by green ash. Symptoms of EAB infestation (reduced canopy, woodpecker attack, and epicormic growth) were observed in all study sites at the start of the study (2015 and 2016), particularly on large ash trees. Based on initial observations, we noted that EAB population density varied among sites and EAB-caused ash mortality was low (<5%) when *T. planipennisi* and/or *S. galinae* were first released. Details of tree basal area, species composition, and relative abundance at each study site are presented in the supplementary data (ST1).

### Parasitoid Rearing and Field Releases

*Tetrastichus planipennisi* released in this study were the progeny ( $F_{30}$ – $F_{50}$ ) of a colony started with wasps collected from northeast



**Fig. 1.** Map of the study region in southern New England and parts of New York, showing the location of the six study sites: CT1, Mattatuck State Forest one in Litchfield Co.; CT2, Mattatuck State Forest two in Litchfield Co.; CT3, Cromwell Wildlife Management Area in Middlesex Co.; MA1, Dalton State Conservation forest in Berkshire Co.; NY1, Red Hook Conservation Forest in Dutchess Co., NY2, Dubac Farmland Wildlife Area in Columbia Co.

China (Liu et al. 2007), whereas the *S. galinae* individuals released were the progeny ( $F_{10}$ – $F_{15}$ ) of a colony started with wasps collected from the Russian Far East (Duan et al. 2012). Both parasitoids were reared at the APHIS EAB Biocontrol Rearing Facility in Brighton, Michigan, on late-instar EAB larvae in small green ash bolts (approx. 14-cm long  $\times$  3- to 8-cm diameter) according to methods published previously (Duan and Oppel 2012, Duan et al. 2014).

To release *T. planipennisi*, we nailed ash bolts containing late-instar parasitoid larvae or pupae to ash trees (approx. 1.5-m above the ground) with signs of EAB infestation. Based on the estimates from the Brighton Rearing facility, each ash bolt contained ~150 *T. planipennisi* larvae and/or pupae (from five parasitized host larvae). For *S. galinae* releases, naïve, mixed-sex adults (<7 d after emergence) were liberated on trunks of ash trees showing apparent signs of EAB infestation (woodpecker feeding, reduced crown condition and/or epicormic growth) at each study site. The timing of releases and numbers of adults released at each of the six study sites are summarized in Table 1 for *T. planipennisi* and Table 2 for *S. galinae*. Briefly, the first releases of *T. planipennisi* were made between June and October of 2015 at each site and repeated between June and August of 2016. Releases of *S. galinae* began with liberation of large numbers (893–903) of adults per site, with releases staggered

over time, with some adults being released every 1–3 wk from June through August of 2016; in 2017, small numbers of *S. galinae* adults (98 adults per site) were also released at each study site during the same period as in 2016. *Tetrastichus planipennisi* and *S. galinae* were released onto the main trunk of 5–15 ash trees <20 m from the center of each study plot. Sex ratios of the released *T. planipennisi* and *S. galinae* were not determined at the field releases; however, progeny of both species reared on EAB larvae are normally female-biased at a 3:1 female-to-male ratio (i.e., 75% female) (Duan and Oppel 2012, Duan et al. 2014).

### Sampling Procedures

Following initial releases of parasitoids at each study site, we measured the frequency of EAB larval mortality factors, including parasitism by *S. galinae* or *T. planipennisi*, woodpecker predation, and parasitism by adventive or native North American parasitoids, using a fall-sampling protocol similar to that used in our previous studies (Duan et al. 2013, Duan et al. 2015a,b, Duan et al. 2017). Each fall (October to November) from 2015 to 2018, we selected three to seven live ash trees (DBH range from 8 to 22 cm) with apparent signs of EAB infestation (e.g., fresh woodpecker feeding, epicormic

**Table 1.** Time, frequency, and number of *T. planipennisi* (adults) releases in 2015 and 2016 at study sites in the northeastern United States

States	Site codes	Release time (year: month)	Frequency of releases	Total no. of adult parasitoids released*
Connecticut	CT1	2015: Aug.–Sept.	3	3,000
		2016: June–Aug.	6	1,918
	CT2	2015: Aug.–Sept.	3	3,000
		2016: June–Aug.	6	1,917
	CT3	2015: Jun.–Oct.	10	16,800
		2016: June–Aug.	6	1,909
Massachusetts	MA1	2015: Aug.–Sept.	3	6,000
	MA1	2016: June–Aug.		2,399
New York	NY1	2015: Aug. – Sept.	3	3,000
	NY1	2016: June – Aug.		1,933
	NY2	2015: Aug. – Sept.	3	3,000
	NY2	2016: June – Aug.	6	1,965

CT1, Mattatuck State Forest one in Litchfield Co.; CT2, Mattatuck State Forest two in Litchfield Co.; CT3, Cromwell Wildlife Management Area in Middlesex Co.; MA1, Dalton State Conservation forest in Berkshire Co.; NY1, Red Hook Conservation Forest in Dutchess Co.; NY2, Dubac Farmland wildlife Area in Columbia Co.

\*Both females and males with approximately 3:1 ratio.

**Table 2.** Time, frequency, and number of *S. galinae* (adults) releases in 2016 and 2017 at study sites in the northeastern United States

State	Site codes	Release time (year: month)	Frequency of releases	Total no. of adult parasitoids released*
CT	CT1	2016: June–Aug.	6	893
		2017: June–Aug.	5	98
	CT2	2016: Aug.–Sept.	6	903
		2017: June–Aug.	5	98
	CT3	2016: June–Aug.	5	903
		2017: June–Aug.	5	98
MA	MA1	2016: June–Aug.	5	771
	MA1	2017: June–Aug.	5	98
NY	NY1	2016: June - Aug	6	903
	NY 1	2017: June – Aug.	5	98
	NY2	2016: June – Aug.	6	893
	NY 2	2017: June – Aug.	5	98

CT1, Mattatuck State Forest one in Litchfield Co.; CT2, Mattatuck State Forest two in Litchfield Co.; CT3, Cromwell Wildlife Management Area in Middlesex Co.; MA1, Dalton State Conservation forest in Berkshire Co.; NY1, Red Hook Conservation Forest in Dutchess Co.; NY2, Dubac Farmland wildlife Area in Columbia Co.

\*Both females and males with approximately 3:1 ratio.

growth) within a radius of  $\approx 100$  m from the parasitoid release area at each study site. Each selected tree was cut and the main trunk and branches ( $>5$  cm in diameter) debarked with a drawknife and examined for the presence of immature stages of EAB and associated parasitoids. Carefully removing both the outer and inner bark tissue, we examined each EAB gallery or pupation chamber (formed by mature J-shaped, fourth-instar larvae) and determined the stage and fate of each larva using methods described in [Duan et al. \(2015b\)](#). Parasitism of EAB larvae by *T. planipennisi* was scored in the field based on the presence of visible parasitoid larvae or pupae, signs of emerged parasitoids (meconium along with small emergence holes on the bark), or adult parasitoids in host galleries. Parasitism by *S. galinae* was scored in the field based on the presence of larval clutches or broods (normally 9–15 larvae per brood) or cocoons (containing overwintering parasitoid larvae, pupae, or pharate adults) and, subsequently in the laboratory, by identification of adults emerging from the field-collected cocoons ([Duan et al. 2014](#)). In addition, all apparently live but not obviously parasitized EAB larvae from field sampling were collected and returned to University of Massachusetts or USDA ARS-BIIR quarantine facility for rearing or dissection to detect any additional cases of parasitism.

In addition to parasitism by *S. galinae* and *T. planipennisi*, we recorded three other categories of mortality associated with EAB larvae or pupae: 1) parasitism by other hymenopteran parasitoids (through field observation, laboratory rearing, and dissection of individual EAB larvae), primarily native *Atanycolus* (Braconidae) species and *Phasganophora sulcata* Westwood (Chalcididae), 2) woodpecker predation, and 3) mortality from undetermined factors (which could be due to host tree resistance, pathogens, weather, or intraspecific competition; [Liu and Bauer 2006](#); [Duan et al. 2015b, 2017](#)).

### Data Analysis

Throughout the study, we also observed some ‘old’ EAB galleries or pupation chambers, which were completely enveloped by the growth of a host tree callus. In most cases, the EAB larvae or pupae inside these ‘old’ galleries or pupation chambers either had exited as adults (evidenced by the ‘old’ D-shaped holes enveloped with the growth of host tree callus) or removed by wood peckers (evidenced by the ‘old’ wood pecker feeding signs enveloped with the growth of host tree callus). Because these ‘old’ galleries had been most likely created by the previous generations of EAB larvae before our sampling time, data on the fate of those larvae or pupae inside the ‘old’ EAB

galleries or pupation chambers (enveloped by host tree callus) were excluded from our data analysis.

We used a general linear model (two-way ANOVA [analysis of variance]) to detect differences in tree DBH and EAB density among study sites, years of sampling or interactions between study sites and years. The EAB density was calculated based on the total number of live, immature larvae, including all instars from the first instar (L1) to mature J-shaped fourth instar (JL), as well as adults that had emerged from the sampled tree (based on observation of the fresh D-shaped exit holes that were not enveloped with host tree callus), and the total phloem area of each sampled tree. The total phloem area ( $y$ ) of each sampled tree was estimated using a second-order polynomial model ( $y = 0.024x^2 - 0.307x + 2.63$ ) as a function of the tree DBH ( $x$ ) (McCullough and Siegert 2007).

To evaluate the establishment of both *S. galinae* and *T. planipennisi* over time, we first calculated the probability, pooled across all the study sites, that a sampled ash tree would have one or more broods of *S. galinae* or *T. planipennisi* in relation to the year of sampling and then analyzed the data with the likelihood-ratio  $\chi^2$  tests. We then used a general linear (ANOVA) model to analyze the abundance (mean number of broods or clutches observed per square meter of sampled phloem area) of *S. galinae* or *T. planipennisi* in relation to study sites and years of sampling. Before analysis, we transformed the parasitoid abundance data with a square root function to normalize the distribution.

Mortality rates due to *S. galinae* or *T. planipennisi* (as well as other resident larval parasitoids) were calculated as marginal attack rates by excluding the EAB larvae killed by either woodpeckers or by undetermined factors (see Elkinton et al. 1992, Duan et al. 2015b) and including only EAB larval stages (third to fourth instars) that were old enough to be subjected to parasitism *T. planipennisi* or *S. galinae* (i.e., excluding first to second instars of host larvae). However, mortality rates due to woodpeckers and undetermined factors were calculated as a proportion of the number of dead individuals from each cause relative to the total number of individuals (dead and live) of all EAB stages. Mortality rates from each of these observed factors were analyzed using the likelihood ratio  $\chi^2$

test based on nominal logistic regression model, which included the effects of study sites, years of sampling and the site-year interactions. All statistical analyses were carried out with JPM 13.01 statistical software (Sall et al. 2017).

## Results

### Recovery and Abundance of Introduced Biocontrol Agents

Parasitism by both *S. galinae* and *T. planipennisi* was observed in ash trees from at least two or more sites in each species' initial release year (*S. galinae* at five sites in 2016 and *T. planipennisi* at two sites in 2015). By the fall of 2018, *S. galinae* parasitism was observed at all study sites, whereas *T. planipennisi* parasitism was observed at four of the six sites (CT1, CT3, NY1, and NY2). Across all the study sites (Fig. 2), the percentage of sampled ash trees with one or more broods of *S. galinae* increased significantly from 30.4% in 2016, the year of its first release, to 60.0% in 2018 (likelihood ratio  $\chi^2 = 23.883$ ;  $df = 3$ ,  $P < 0.0001$ ). In contrast, the percentage of sampled ash trees containing one or more *T. planipennisi* broods (Fig. 2) only increased slightly from 20.0% in 2015, the first year of release to 28.0% in 2018 (likelihood ratio  $\chi^2 = 2.074$ ;  $df = 3$ ,  $P < 0.5571$ ).

The mean number of *S. galinae* broods per square meter of phloem area from a sampled tree increased 10- to 20-fold at the three Connecticut sites (CT1, CT2, and CT3) and 1.5-fold at one New York site (NY1), from  $<1.8$  brood/m<sup>2</sup> of phloem at the first year of release (2016) to 2.7–14.3 broods 2 yr after the first release (2018), whereas the mean number of broods of *S. galinae* per square meter of phloem area of sampled ash trees remained less than one in 2018 at one New York site (NY2) and the Massachusetts site (Fig. 3A). In contrast, the mean number of *T. planipennisi* broods per square meter phloem area of a sampled tree remained low (0–2.3 broods) across all the study sites during the entire 4-yr study period (Fig. 3B). ANOVA detected a highly significant effect of year of sampling on the number of broods of *S. galinae* per square meter of phloem area ( $F = 8.7066$ ;  $df = 3, 93$ ;  $P < 0.0001$ ) but did not detect any significant overall effect of study sites ( $F = 1.8905$ ;  $df = 3, 93$ ;

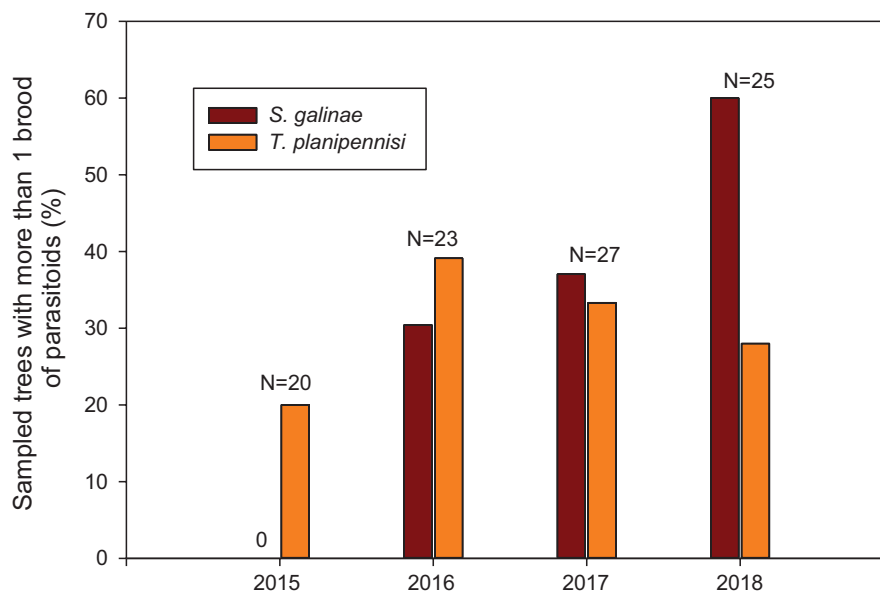
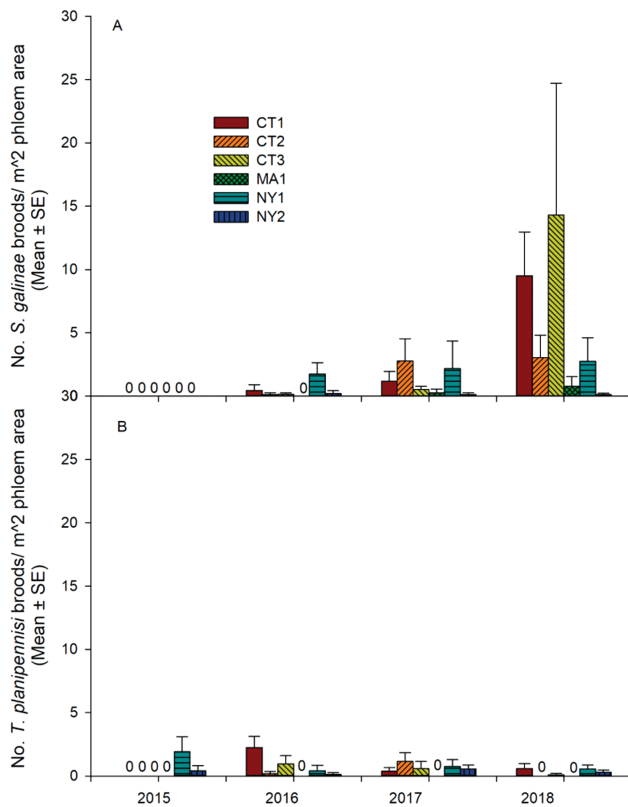


Fig. 2. Percentage of sampled ash trees with one or more broods of *S. galinae* or *T. planipennisi* across all the six study sites each year following parasitoid releases. Numbers above each bar represent total number of trees ( $n$ ) sampled at each site.



**Fig. 3.** Mean ( $\pm$ SE) number of broods of *S. galinae* (A) and *T. planipennisi* (B) observed per square meter of phloem area in a sampled tree at each study site and sampling time (year) following parasitoid releases. Zeros represent no observation of either *S. galinae* or *T. planipennisi* broods.

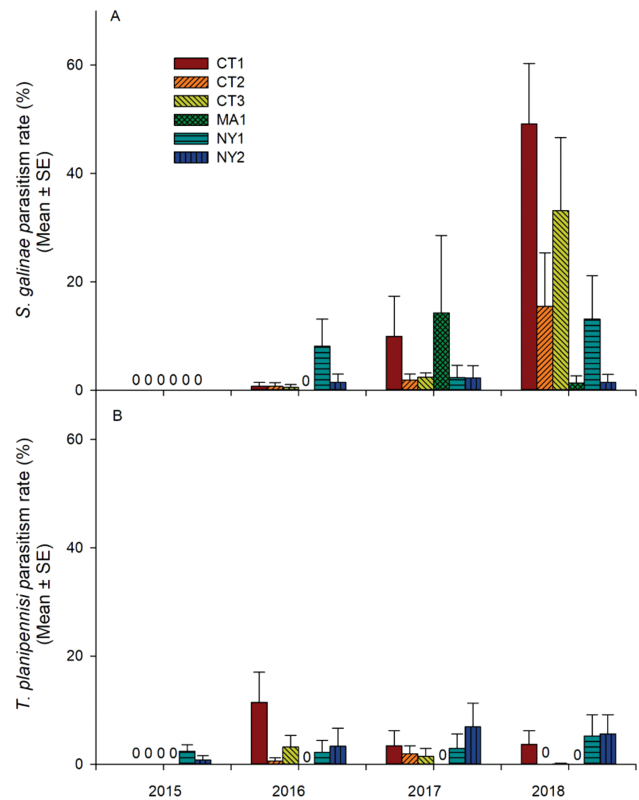
$P = 0.1205$ ), and nor was the interaction between sampling year and study site significant ( $F = 1.6955$ ;  $df = 3, 93$ ;  $P = 0.0746$ ). The mean number of *T. planipennisi* broods observed per tree did not vary significantly with year of sampling ( $F = 1.4623$ ;  $df = 3, 93$ ;  $P = 0.2385$ ) but did so significantly with study site ( $F = 3.9287$ ;  $df = 5, 93$ ;  $P = 0.0061$ ), which also interacted significantly with the year of sampling ( $F = 2.0372$ ;  $df = 15, 93$ ;  $P = 0.0264$ ).

### Rate of Parasitism by the Introduced Biocontrol Agents

In the first year of release (2016), low (<1.5%) parasitism of EAB larvae by *S. galinae* was observed at the three Connecticut sites and the two New York Sites, whereas no parasitism was observed at the Massachusetts site (Fig. 4A). For *T. planipennisi* in 2016, only low parasitism rates (<1.5%) were observed at the two New York sites, and no parasitism was observed at the other four sites in 2015.

By fall of 2018, parasitism rates of *S. galinae* increased nearly 30-fold to 33.1–49.2% at the two Connecticut sites (CT1 and CT3) and approximately 13-fold to 13.1–15% at the other Connecticut site (CT2) and one of the New York sites (NY1). In contrast, *S. galinae* parasitism rates remained low (<1.5%) at the other New York site (NY2) and the Massachusetts site (MA1). In contrast, no parasitism by *T. planipennisi* was observed at MA1 throughout the study period, nor at CT2 in 2018, whereas in 2018 low parasitism rates (0.1–5.6%) by *T. planipennisi* were observed at the remaining four study sites (CT1, CT3, NY1, and NY2).

Nominal logistic regression analyses indicated that parasitism rate by *S. galinae* increased highly significantly with sampling years ( $\chi^2 = 32.0258$ ;  $df = 3$ ;  $P < 0.0001$ ) but did not vary significantly



**Fig. 4.** Percent parasitism (marginal attack rate) of EAB larvae by *S. galinae* (A) and *T. planipennisi* (B) at each of the study site and sampling time (year) following parasitoid releases. Zeros indicate no observation of either *S. galinae* or *T. planipennisi* parasitism.

among study sites ( $\chi^2 = 1.3702$ ;  $df = 5$ ;  $P = 0.9276$ ), although there was a highly significant interaction between study site and sampling year ( $\chi^2 = 72.9170$ ;  $df = 15$ ;  $P < 0.0001$ ). In contrast, parasitism by *T. planipennisi* was only marginally affected by sampling year ( $\chi^2 = 7.6790$ ;  $df = 3$ ;  $P = 0.0531$ ), but it varied significantly among study site ( $\chi^2 = 592.8658$ ;  $df = 5$ ;  $P < 0.0001$ ). In addition, there was a significant interaction for *T. planipennisi* parasitism between year and study site ( $\chi^2 = 33.7121$ ;  $df = 15$ ;  $P = 0.0023$ ).

### EAB density and mortality from factors other than introduced biocontrol agents

There were significant differences in the average DBH (7.8–14.9 cm) of sampled ash trees among the six study sites ( $F = 4.0611$ ;  $df = 5, 94$ ;  $P = 0.0050$ ), but not among different years of sampling ( $F = 0.2308$ ;  $df = 3, 94$ ;  $P = 0.7954$ ), nor was there any interaction between the study site and year on tree DBH ( $F = 1.220$ ;  $df = 15, 94$ ;  $P = 0.3544$ ). Across all the study sites, mean EAB density (number of alive stages per square meter of phloem) ranged from 3.5 to 64.5 during the 4-yr study period (Table 3). ANOVA detected highly overall significant differences in EAB densities among study sites ( $F = 4.9003$ ;  $df = 5, 94$ ;  $P = 0.0015$ ) and significant interactions between the study site and year ( $F = 3.8075$ ;  $df = 15, 94$ ;  $P < 0.0001$ ). However, we observed no overall significant difference in average EAB densities among different years ( $F = 0.7841$ ;  $df = 3, 94$ ;  $P = 0.4769$ ).

Across all the study sites, woodpeckers caused an average of 4.3–48.1% mortality of the observed immature EAB stages during the 4-yr study period, whereas the undetermined factors killed 1.2–29.3% of immature EAB stages, and resident parasitoids other than

**Table 3.** Mean ( $\pm$ SE) DBH of sampled trees, EAB density, and mortality caused by woodpeckers, undetermined factors, and resident parasitoids other than released biocontrol agents (*S. galinae* and *T. planipennisi*)

Sites	Years	No. of trees sampled	DBH (cm) of sampled trees	EAB density (n)/m <sup>2</sup> phloem	% EAB killed by woodpeckers	% EAB killed by undetermined factors*	% EAB killed by resident parasitoids
CT1	2015	4	8.99 $\pm$ 1.79	15.25 $\pm$ 4.70	20.14 $\pm$ 2.52	8.41 $\pm$ 2.19	0.82 $\pm$ 0.82
	2016	4	10.03 $\pm$ 1.42	21.97 $\pm$ 9.15	27.43 $\pm$ 10.38	9.15 $\pm$ 27.43	0.65 $\pm$ 0.41
	2017	5	9.08 $\pm$ 0.67	10.38 $\pm$ 3.44	15.59 $\pm$ 3.54	3.44 $\pm$ 15.59	0.0 $\pm$ 0.0
	2018	5	9.37 $\pm$ 0.79	11.89 $\pm$ 2.19	5.95 $\pm$ 3.22	2.19 $\pm$ 5.95	0.43 $\pm$ 0.43
CT2	2015	3	8.77 $\pm$ 1.01	49.08 $\pm$ 17.53	24.66 $\pm$ 8.16	17.53 $\pm$ 24.66	0.14 $\pm$ 0.14
	2016	3	12.23 $\pm$ 1.59	13.54 $\pm$ 1.91	4.32 $\pm$ 2.27	1.91 $\pm$ 4.32	0.0 $\pm$ 0.0
	2017	4	9.15 $\pm$ 0.55	64.48 $\pm$ 16.27	13.87 $\pm$ 4.03	16.27 $\pm$ 13.87	0.0 $\pm$ 0.0
	2018	4	10.39 $\pm$ 2.15	8.01 $\pm$ 2.75	15.83 $\pm$ 15.43	2.75 $\pm$ 15.83	0.26 $\pm$ 0.26
CT3	2016	4	9.85 $\pm$ 1.03	16.45 $\pm$ 1.53	26.38 $\pm$ 7.23	1.53 $\pm$ 26.38	0.42 $\pm$ 0.42
	2017	4	9.03 $\pm$ 1.04	17.16 $\pm$ 6.53	47.87 $\pm$ 12.77	6.53 $\pm$ 47.87	1.45 $\pm$ 1.13
	2018	4	11.33 $\pm$ 1.01	15.95 $\pm$ 8.73	34.82 $\pm$ 12.73	8.73 $\pm$ 34.82	0.11 $\pm$ 0.11
MA1	2015	7	13.57 $\pm$ 1.55	10.55 $\pm$ 3.45	47.65 $\pm$ 4.54	3.45 $\pm$ 47.65	0.33 $\pm$ 0.24
	2016	4	9.75 $\pm$ 1.29	5.58 $\pm$ 2.70	48.12 $\pm$ 13.62	2.70 $\pm$ 48.12	0.0 $\pm$ 0.0
	2017	5	12.12 $\pm$ 2.01	3.48 $\pm$ 1.51	27.04 $\pm$ 10.48	1.51 $\pm$ 27.04	0.0 $\pm$ 0.0
	2018	4	14.93 $\pm$ 2.65	50.43 $\pm$ 28.08	23.20 $\pm$ 7.79	28.08 $\pm$ 23.20	0.10 $\pm$ 0.10
NY1	2015	3	8.80 $\pm$ 1.68	55.83 $\pm$ 21.19	30.72 $\pm$ 17.28	21.19 $\pm$ 30.72	3.31 $\pm$ 1.92
	2016	4	9.60 $\pm$ 1.99	19.14 $\pm$ 8.59	26.80 $\pm$ 1.98	8.59 $\pm$ 26.80	3.85 $\pm$ 2.50
	2017	5	11.62 $\pm$ 1.06	31.89 $\pm$ 5.56	12.31 $\pm$ 5.05	5.56 $\pm$ 12.31	1.32 $\pm$ 0.56
	2018	4	7.78 $\pm$ 0.68	10.18 $\pm$ 2.67	43.22 $\pm$ 12.21	2.80 $\pm$ 43.22	1.76 $\pm$ 1.76
NY2	2015	3	9.77 $\pm$ 1.69	22.34 $\pm$ 2.70	42.81 $\pm$ 0.69	2.70 $\pm$ 42.81	0.97 $\pm$ 0.97
	2016	4	9.93 $\pm$ 0.82	12.16 $\pm$ 5.75	32.48 $\pm$ 3.76	5.75 $\pm$ 32.48	0.0 $\pm$ 0.0
	2017	4	8.65 $\pm$ 0.68	7.24 $\pm$ 2.08	27.80 $\pm$ 8.63	2.08 $\pm$ 27.80	0.56 $\pm$ 0.56
	2018	4	9.20 $\pm$ 0.70	13.26 $\pm$ 1.85	40.07 $\pm$ 7.35	1.85 $\pm$ 40.07	0.0 $\pm$ 0.0

\* including putative host tree resistance, insect pathogen, weather, and/or intraspecific competitions.

the two introduced biocontrol agents parasitized 0–3.9% of EAB larvae during the same period (Table 3). The resident (native and adventive) parasitoids consisted primarily of two groups of native North American species (*Atanycolus* spp. and *P. sulcata*), with some minor contribution by vagrant (adventive) species such as *Balcha indica* Mani and Kaul (Eupelmidae) and an unidentified braconid wasp. Logistic regression analyses detected highly significant effects of study sites on EAB mortality caused by woodpeckers (likelihood ratio  $\chi^2 = 194.8861$ ; df = 5, 93;  $P < 0.0001$ ), undetermined factors (likelihood ratio  $\chi^2 = 65.9720$ ; df = 5;  $P < 0.0001$ ), and the resident parasitoids ( $\chi^2 = 60.3381$ ; df = 5;  $P < 0.0001$ ). Sampling years had a significant effect on the mortality caused by undetermined factors ( $\chi^2 = 17.9434$ ; df = 3;  $P = 0.0005$ ) but not on the mortality by woodpeckers ( $\chi^2 = 3.3359$ ; df = 3;  $P = 0.3472$ ), nor by the resident parasitoids ( $\chi^2 = 4.6937$ ; df = 3;  $P = 0.1956$ ). In addition, there were significant interactions between study sites and sampling years on all these observed mortalities (likelihood ratio  $\chi^2$  tests, all Type I errors  $< 0.002$ ).

## Discussion

Findings from this 4-yr field study provide the first evidence that *S. galinae* can establish and attack a large portion of EAB larvae in pole size trees (the size we sampled) in ash-dominated forests in the northeastern United States. Our data also showed that *S. galinae* established self-sustaining populations in all six release sites and its population density increased 1.5- to 20-fold at four of the release sites within 2 yr of the principal year of release, whereas *T. planipennisi* established at only four of the six study sites and its population density was much lower than *S. galinae*. In addition to parasitism by these two introduced larval parasitoids, results from this study also showed that EAB populations in this region suffered low (<5%) to moderate (<50%) mortality from attack by

woodpeckers, undetermined factors (host tree resistance, diseases, and weather), and resident (adventive or native North American) parasitoids. These findings are relevant and applicable to the development of the biocontrol-based EAB management plan to protect saplings and surviving ash trees capable of reaching maturity in North America.

Climate-matching analysis indicates that the northern United States and part of southern Canada have a high climate match to the Russian Far East (USDA APHIS 2015), a region where *S. galinae* is the most abundant parasitoid of EAB larvae and *T. planipennisi* is the least abundant larval parasitoid (Duan et al. 2012, 2018). In addition, the upper midwestern United States (Michigan and Wisconsin) appears to have a high level of climate-match to northeastern China (USDA APHIS 2007), a region where *T. planipennisi* is the most abundant EAB larval parasitoid and where *S. galinae* has not been observed (Wang et al. 2016, Duan et al. 2018). The fact that both *S. galinae* and *T. planipennisi* established self-sustaining populations in all or most of the six study sites in our study region 2 yr after their first large releases suggests that the region's climatic conditions are suitable for the reproduction and overwintering of both parasitoids. We suspect that differences in site-specific factors such as the phenology of EAB larval stages, ash tree species and size, and the reproductive biology of the agents may have contributed to the variation in the establishment or abundance of *S. galinae* and *T. planipennisi* among our different study sites. For example, the MA1 site had only white ash, with an average DBH range from 10.4 to 14.9 cm, larger than the ash trees at the other five sites, which had either both white ash and green ash or green ash alone, with a lower average DBH of 8.8–12.3 cm. It is well documented that *T. planipennisi* is less effective in attacking EAB larvae in large trees (DBH >12 cm) because of its short ovipositor (Abell et al. 2012), whereas *S. galinae* can attack EAB larvae infesting much larger ash trees (Duan et al. 2012,

Murphy et al. 2017). The difference in the size of EAB-infested host trees among different study sites may in part explain variation among sites in parasitism rates and recovery of *S. galinae* and *T. planipennisi*. In addition, we also suspect that the phenology of EAB larvae (by instars) may have varied among our study sites. Unfortunately, this variation was not directly assessed before or during parasitoid release. Future parasitoid releases against EAB could be improved if phenology of the EAB at the release site was assessed to verify that suitable pest stages (third- or fourth-instar larvae) were present at or before parasitoid liberations.

Previous field surveys in the EAB's native range showed that *S. galinae* parasitized a mean of 62.7% of late instars of EAB larvae in the Vladivostok area of the Russian Far East (Duan et al. 2012), whereas *T. planipennisi* caused as high as 44% parasitism of late-instar EAB larvae in Northeast China (Liu et al. 2007, Wang et al. 2016). The levels of *S. galinae* and *T. planipennisi* parasitism observed in our study are lower than those from the two parasitoids' native ranges; however, the high density (3–14 brood/m<sup>2</sup> of phloem area) and parasitism (13.1–49%) of *S. galinae* 2 yr after its principal release at our study sites strongly suggests that *S. galinae* may be a successful biocontrol agent for suppression of EAB to protect surviving ash trees capable of reaching maturity in the northeastern United States. In addition, previous studies showed that multi-parasitism of EAB larvae by both *S. galinae* and *T. planipennisi* was rather low (<5%) even under the optimal laboratory rearing condition for intrinsic competition (Yang et al. 2012, Wang et al. 2015). After examining the effect of various extrinsic factors (e.g., host densities, host attack rates, and the size of parasitoid progeny broods) on the potential competition between *S. galinae* and *T. planipennisi*, Wang et al. (2015) concluded that the two species EAB parasitoids can co-exist through trade-offs in their parasitism efficiency and brood sizes. Findings from these previous studies as well as our current study strongly suggest that both *S. galinae* and *T. planipennisi* can coexist in the same release areas and thus provide necessary protection of surviving ash saplings and trees capable of reaching maturity against EAB in North America.

Currently, we have little knowledge of the rate of spread of *S. galinae*, and the sampling protocol used in this study focused only on the recovery of the released parasitoids from EAB infested trees within the area (≈100 m radius) immediately adjacent to release points at each study site. However, previous studies with *T. planipennisi* in Michigan and New York found that *T. planipennisi* can spread up to 3 km/yr (Duan et al. 2013, Jones et al. 2019). Considering the larger body and wings of *S. galinae*, we speculate that it may spread even faster than *T. planipennisi* in the field. Future studies on the spread and dispersal patterns of *S. galinae* from areas with newly established populations could help optimize establishment of this species and increase its efficacy in controlling EAB populations.

Besides parasitism by *S. galinae* and *T. planipennisi*, in our study plots EAB larvae also suffered mortality from woodpeckers, undetermined factors (such as host tree resistance, diseases, weather, and/or intraspecific competition), and other resident larval parasitoids in our study sites. Among these factors, woodpeckers were the most abundant over the 4-yr study period, removing 4–48% of various EAB stages from feeding galleries and/or pupal chambers. Undetermined factors killed 1.2–29% of immature EAB stages across different sites during the study period. Low levels (0–3.8%) of parasitism by resident (nonreleased) larval parasitoids (primarily *Atanycolus* spp. and *P. sulcata*) were observed at different sites over the 4-yr study period. Previous studies in Maryland and

Michigan have reported similar levels of EAB larval mortality from woodpecker attacks and undetermined factors (Duan et al. 2015b, Jennings et al. 2016). Low levels (<5%) of parasitism of EAB larval by adventive or native North American parasitoids have also been reported in field studies in Maryland, Pennsylvania, Kentucky, Virginia, and Tennessee (e.g., Duan et al. 2009, Spinos et al. 2014, Davidson and Rieske 2015, Hooie et al. 2015, Jennings et al. 2016). However, field studies in Michigan showed that one group of native North American braconids, *Atanycolus* spp., sometimes parasitized up to 60% of late EAB larvae in some infested ash stands during the outbreak phase of the pest (Cappaert and McCullough 2009, Duan et al. 2015b). Currently, we have little information on how these other mortality factors interact with the introduced *S. galinae* and/or *T. planipennisi* in suppressing EAB populations. It is plausible that these resident mortality factors could help provide biological community resistance to EAB invasion and thus complement the role of the introduced biological control agents in regulating the pest's population dynamics (e.g., Murphy et al. 2018). Future research addressing this question has been planned in our study plots, where *S. galinae* and *T. planipennisi* have spread and established self-sustaining populations.

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