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Successful establishment, spread, and impact of the introduced parasitoid *Spathius galinae* (Hymenoptera: Braconidae) on emerald ash borer (Coleoptera: Buprestidae) populations in postinvasion forests in Michigan

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Spathius galinae is a larval parasitoid native to the Russian Far East that was approved for release in the United States in 2015 for biological control of the emerald ash borer (EAB), *Agrilus planipennis*, an invasive beetle from Asia responsible for widespread mortality of ash trees (*Fraxinus* spp.) in North America. From 2015 to 2017, 1,340–1,445 females of *S. galinae* along with males were released into each release plot, paired with a nonrelease control plot (1–12.5 km apart), at 6 postinvasion forested sites containing abundant pole-sized ash trees in Michigan. By 2018, *S. galinae* had spread to all but one control plot. Based on the first year that *S. galinae* was found in trees in each control plot and the distances of those trees to the parasitoid release point within each site, we estimated that *S. galinae* spread at 3.7 (±1.9) km per year after its initial releases in 2015. The proportion of sampled trees with *S. galinae* broods, brood densities within sampled trees, and parasitism of EAB larvae increased sharply in both control and release plots after the last field releases in 2017, with the highest parasitism rates (42.8–60.3%) in 2020. Life table analysis showed that *S. galinae* alone reduced EAB's net population growth rate by 35–55% across sites from 2018 to 2020. These results demonstrate that *S. galinae* has established an increasing population in Michigan and now plays a significant role in reducing EAB populations in the area.

Key words: invasive, woodborer, parasitoid, classical biological control, life table analysis

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive phloem-feeding beetle native to Asia that was first discovered in southern Michigan, United States, and nearby Ontario, Canada in 2002 (Herms and McCullough 2014). This pest causes widespread mortality of ash trees (*Fraxinus* spp.) in North America and is now established in 36 US states, Washington D.C., and 5 Canadian provinces (Canadian Food Inspection Agency 2023, Emerald Ash Borer Information 2023). In Europe, this pest was first detected in Moscow in 2003 and has since spread throughout European Russia and eastern Ukraine, causing widespread mortality of native European ash trees where it was established in these regions (Orlova-Bienkowskaja and Bieńkowski 2022). Early attempts to eradicate EAB by both United States and Canadian regulatory agencies were implemented but quickly abandoned as the beetle spread rapidly, was difficult to detect, and became too widespread (GAO 2006). By 2020, federal quarantine regulations that restricted the movement of EAB-infested wood or plant materials in the United States were also discontinued (Federal Register 2020).

Ongoing efforts of research, development, and implementation of management strategies against this invasive pest were subsequently redirected towards biological control, insecticide treatment or physical destruction of infested trees, and the development of resistant ash genotypes (e.g., Liu et al. 2007, Bauer et al. 2015, Koch et al. 2015, McCullough et al. 2015, Mercader et al. 2015, Duan et al. 2018). Although highly effective systemic insecticides are available to protect high value, landscape ash (Herms et al. 2009, Sadof et al. 2021), costs and environmental concerns prevent the widespread use of chemical controls against EAB in natural forests. In contrast, biological control via release and establishment of specialized natural enemies from EAB's native range has become the most effective option for areawide management of this pest in natural forests of North America (Bauer et al. 2015, Duan et al. 2018, 2023).

Soon after the EAB was detected in Michigan, a classical biocontrol program against the pest was developed by the U.S. Department of Agriculture (USDA). This program was first implemented in 2007 with regulatory approvals for environmental releases of 3 hymenopteran parasitoids from northeast China: *Tetrastichus planipennisi* Yang (Eulophidae), *Spathius agrili* Yang (Braconidae), and *Oobius agrili* Zhang and Huang (Encyrtidae). These introduced biocontrol agents were first released between 2007 and 2010 in Michigan and later in other infested states (Bauer et al. 2015). To date, both the egg parasitoid *O. agrili* and the larval parasitoid *T. planipennisi* are well established in many release areas in the United States and are spreading naturally to new areas (Duan et al. 2013, 2015a, Abell et al. 2014, Jennings et al. 2016, 2018, Jones et al. 2019). In some of the early release sites in Michigan, *T. planipennisi* caused high rates of parasitism (30–85%) to late instar (third to fourth instar) EAB larvae in ash saplings (<6 cm diameter at breast height, DBH) 5–7 yr after release (Duan et al. 2017, Gould et al. 2022). However, the relatively short ovipositor (<2.5 mm) of *T. planipennisi* limits its ability to reach EAB larvae in larger (≥12 cm diameter) tree trunks (at breast height) or branches (at the base) with bark more than 3.5 mm thick (Abell et al. 2012, 2016). In addition, the successful establishment and host attack of *T. planipennisi* is also influenced by overwintering phenology of the EAB and climatic conditions in North America (Gould et al. 2020). Parasitism rates by the introduced egg parasitoid *O. agrili* vary from 1 to 32% across different release areas in the United States (Abell et al. 2014, 2016, Davidson and Rieske 2016, Jennings et al. 2018), and its ability to reach EAB eggs appears to be unrelated to the tree bark thickness. However, the introduced larval parasitoid *S. agrili* with a relatively larger body size and longer ovipositor than that of *T. planipennisi* has not established well in most release sites surveyed in the United States (MapBioControl 2023). The failure of *S. agrili* to establish in many northern regions was possibly due to the asynchronization of adult parasitoid emergence in mid-summer with the presence of suitable stages of EAB larvae in spring and fall (Jones et al. 2020).

In the summer of 2015, *Spathius galinae* Belokobylskij & Strazenac (Hymenoptera: Braconidae), a gregarious larval parasitoid from the Russian Far East (part of EAB's native range), was approved for release in the United States (Belokobylskij et al. 2012, Duan et al. 2012, 2015b, Federal Register 2015). This braconid wasp has an ovipositor that is 50% longer than that of its congener *S. agrili* and about twice as long as that of *T. planipennisi*, allowing it to attack larvae in larger, reproductive-age trees (Murphy et al. 2017). Recent field studies in several northeast states (Connecticut, Massachusetts, and New York) and Maryland show that *S. galinae* is successfully established in most of the release sites and has spread 14–90 km from the release points after 3–5 yr (Duan et al. 2019, 2020, Aker et al. 2022, Quinn et al. 2022). Recent life table analyses also showed that *S. galinae* alone caused an average of 31–57% reduction in the net population growth rates of the EAB from 6 ash-dominated forests in 3 northeast states, where the introduced biocontrol agent was released 3–5 yr earlier (Duan et al. 2022, 2023).

Immediately after regulatory approval in 2015, we began releasing *S. galinae* at 6 ash-dominated aftermath forests in southern Michigan, where two of the earlier introduced agents *T. planipennisi* and *O. agrili* had been released between 2007 and 2010 and successfully established self-sustaining populations (Abell et al. 2012, 2014, Duan et al. 2013, 2015a). *Spathius agrili* was also released at these 6 sites but never established (Duan et al. 2013, 2023). Previous studies at these biocontrol sites demonstrated the ability of *T. planipennisi* to protect ash saplings and small trees from EAB-caused mortality (Duan et al. 2017, 2023). Release and establishment of *S. galinae* in these aftermath or postinvasion forests was intended to increase successful attacks on a large portion of EAB larvae located in pole size or larger ash trees with DBH (>12.00 cm) at depths beyond the limit for *T. planipennisi* in attacking EAB.

The objectives of this study were to determine establishment, spread, abundance, and impacts of *S. galinae* for multiple years after its releases in postinvasion ash forests; estimate the mortality of EAB larvae caused by *S. galinae*, woodpeckers, undetermined factors (such as tree resistance, disease, intraspecific competition, and/or weather), and other larval parasitoids, including native North American parasitoids and the previously-introduced agent *T. planipennisi;* and construct EAB life tables to quantify the impact of *S. galinae* on the pest population growth rate.

Materials and Methods

Study Sites

The study was conducted in 6, mixed hardwood bottomland forests (sites) in southern Michigan (Fig. 1), where previous releases of introduced biocontrol agents (*T. planipennisi*, *S. agrili*, and *O. agrili*) were made from 2007 to 2010 (Duan et al. 2013, 2015a, Bauer et al. 2015). At all six of these sites, both *T. planipennisi* and *O. agrili* established abundant populations (Duan et al. 2013, Abell et al. 2014), and EAB population densities declined significantly from initial outbreak densities at these sites since 2012 (Duan et al. 2015a). Many green ash (*Fraxinus pennsylvanica* Marsh.) or white ash (*F. americana* L.) saplings and pole-size trees at these sites appear to be recovering or regenerating and growing in the aftermath of EAB's initial outbreak in this region (from 2009 to 2010), although many ash trees are still being attacked (Duan et al. 2017, Kashian et al. 2018).

These 6 study sites are located across 3 Michigan counties (Ingham, Gratiot, and Shiawassee) and have the characteristics of early-successional, secondary-growth northern deciduous forests, where live small and pole-size green and white ash trees were still abundant but frequently infested with EAB when *S. galinae* was released. The site locations and tree species composition are described in Duan et al. (2013). Briefly, there are 3 sites in Ingham Co.: (i) Central Park and Nancy Moore Park (CP-NM), (ii) Legg Park and Harris Nature Center (LP), and (iii) William M. Burchfield Park (BF); 2 sites in Gratiot Co.: (iv) Gratiot Saginaw State Game Area (GSW) and (v) Maple River State Game Area (MRE); and one in Shiawassee Co.: (vi) Rose Lake State Wildlife Area (RL). Each site is composed of 2 forest plots – one for *S. galinae* release and the other for nonrelease control. Both release and nonrelease control plots for each site were the same ones used in previous studies (Duan et al. 2013, 2015a). The distance between any of 2 study sites varies from 6 to 65 km, while the distance between the parasitoid release and nonrelease plot within a study site ranges from 1.0 to 12.5 km (Fig. 1).

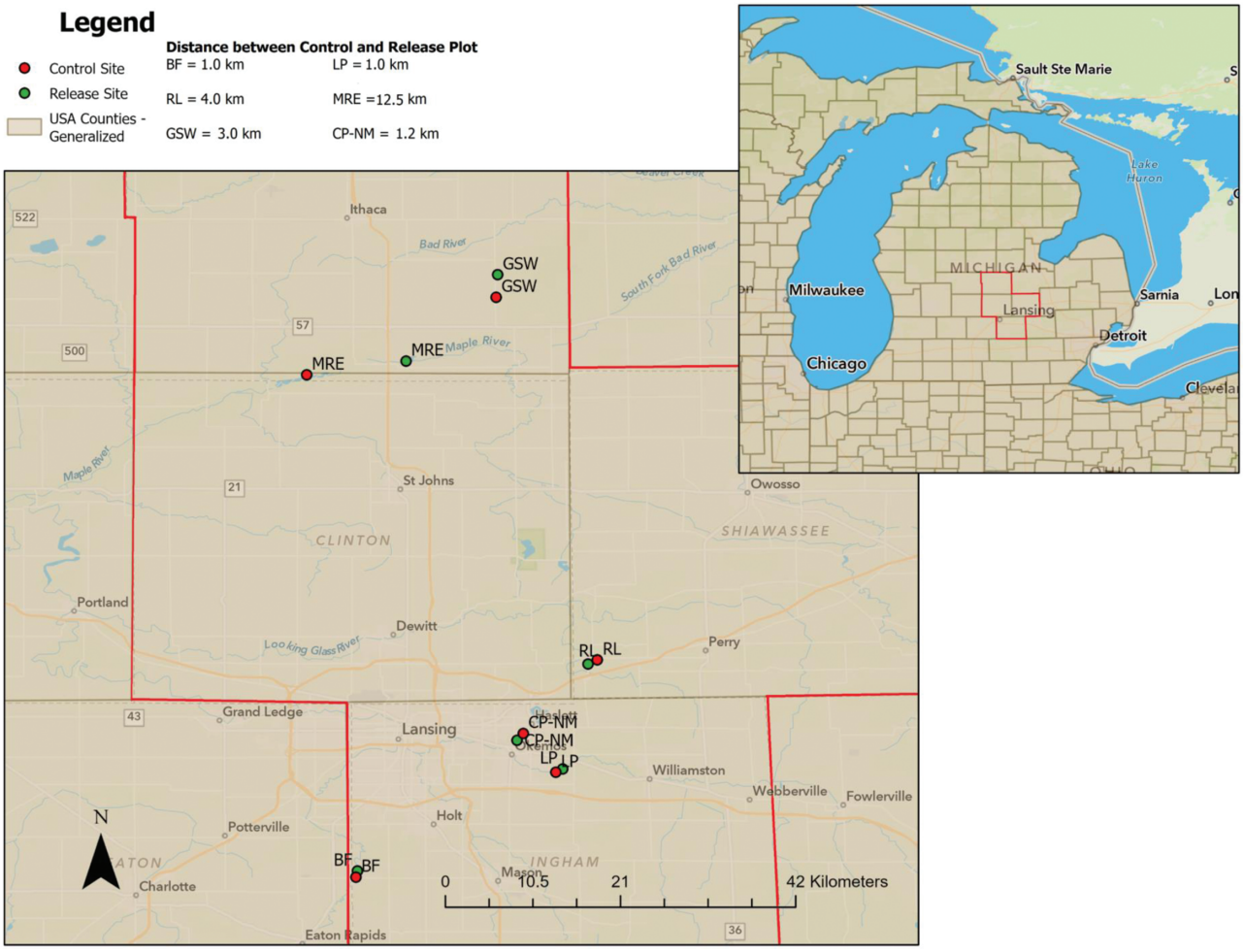

Fig. 1. Map of Michigan showing the locations of 6 *S. galinae* study sites and treatment plots. Study sites included BF = Williams F. Burchfield Park; CP-NM = Central Park and Nancy Moore Park; LP = Legg Park and Harris Nature Center; GSW = Gratiot-Saginaw State Game Area; MRE = Maple River State Game Area; RL = Rose Lake State Wildlife Area.

Parasitoid Rearing and Field Releases

All adults of *S. galinae* released for this study were progeny (generations F_{20} to F_{35}) of parasitoids originally collected from the Primorsky Krai (Vladivostok area) of Russia (Duan et al. 2012). The parasitoids were laboratory-reared on late-instar EAB larvae reared in tropical ash [*Fraxinus uhdei* (Wenzig) Lingelsh] or green ash bolts at the USDA ARS Beneficial Insect Introduction Research Unit (USDA ARS-BIIRU) (Newark, Delaware) or the USDA APHIS Emerald Ash Borer Biocontrol Laboratory (Brighton, Michigan), according to methods described in Duan et al. (2014a).

Before the parasitoids were released, female and male *S. galinae* adults were held together inside rearing cages for 7–10 days to allow for mating and fed with clover honey streaked on the cage screens in the laboratory (at a photoperiod of 16:8 (L:D) h and 25 ± 1.5 °C). The timing of parasitoid releases and numbers of adult females released at each of 6 study sites are summarized in Table 1. Male *S. galinae* adults were also included each time female adults were released at the sites, but males were not counted. Briefly, the first releases of female *S. galinae* at all the study sites (410–440 females per site) were made during July and August of 2015. Releases at each site continued in the summer and fall of 2016 and 2017 with each site receiving a total of 1,340–1,445 female *S. galinae* adults by the end of September 2017 (Table 1). *Spathius galinae* adults were released 1–1.7 m above the ground on trunks of 5–10 ash trees at each release site, with trees being clustered within a radius of 100 m from the center of each release plot.

Sampling Procedures

To determine parasitism rates of EAB larvae by *S. galinae* or other resident larval parasitoids, we felled 4 to 6 live ash trees (9.28–12.86 cm average DBH) with symptoms of EAB infestation (e.g., fresh woodpecker feeding and epicormic growth) in each release and control plots at each study site in the early spring (April to early May) of 2016, 2017, 2018, 2019, and 2021 before adults of EAB or its larval parasitoids (from the previous year) emerged. This early-spring sampling scheme measured the populations of EAB and associated parasitoids from the previous year. Sampling was not carried out in the spring of 2020 (to measure the 2019 EAB and associated parasitoid populations) due to the COVID-19 pandemic. Felled trees, including the main trunk and branches >3 cm in diameter, were debarked with a drawknife and examined for the presence of immature stages (various instars of larvae and pupae) of EABs and associated parasitoids. Parasitism of EAB larvae by *S. galinae* was scored in the field based on the presence of visible stages of the parasitoid larvae (cocooned or noncocooned larvae, cocooned pupae, and/or emerging adults) in host galleries and were confirmed through laboratory rearing of field-collected larvae to adult wasps, which were

Table 1. Time, frequency, and numbers of *Spathius galinae* adult females released at the emerald ash borer biocontrol study sites in southern Michigan from 2015 to 2017

County	Study sites[a]	Release time (year: month)	No. releases	No. of females released
Ingham	BF	2015: Jul–Aug	3	440
		2016: Jun–Sep	12	386
		2017: Jun–Aug	3	567
	CP-NM	2015: Jul–Aug	3	410
		2016: Jun–Sept	14	484
		2017: Jun–Sep	3	565
	LP	2015: Jul–Aug	3	420
		2016: Jun–Sep	14	471
		2017: Jun–Sep	3	551
Gratiot	GSW	2015: Jul–Aug	3	410
		2016: Jun–Aug	12	364
		2017: Jun–Sep	3	566
	MRE	2015: Jul–Aug	3	410
		2016: Jun–Aug	12	378
		2017: Jun–Sep	3	569
Shiawassee	RL	2015: Jul–Aug	3	410
		2016: Jun–Sep	11	408
		2017: Jun–Aug	3	577

[a]BF = Burchfield Park; CP-NM = Central Park-Nancy Moore Parks; LP = Legg Park-Harris Nature Center; GSW = Gratiot Saginaw State Game Area; MRE = Maple River State Game Area; RL = Rose Lake State Wildlife Area. Adult males were also released each time females were released at the sites but were not counted.

identified using voucher *S. galinae* specimens deposited at USDA ARS BIIRU (Duan et al. 2019). In addition, EAB larvae were also collected and returned to either the USDA FS-NRS laboratory or the USDA ARS-BIIRU quarantine facility for rearing and dissection to detect other cases of parasitism.

In addition to parasitism by *S. galinae*, we recorded 3 other categories of mortality associated with EAB larval stages: (i) woodpecker predation, (ii) mortality due to undetermined factors such as intraspecific competition, host tree resistance, unknown diseases, and/or weather (e.g., Liu and Bauer 2006, Duan et al. 2012, 2020), and (iii) parasitism by other (resident) hymenopteran parasitoids, primarily the earlier introduced agent *T. planipennisi* and the native North American hymenopterans: *Atanycolus* spp. (Braconidae) and *Phasgonophora sulcate* Westwood (Chalcididae). Parasitism rates of EAB larvae by *S. galinae* and *T. planipennisi* were calculated according to procedures described in Duan et al. (2022), which excluded EAB larvae preyed upon by woodpeckers, those apparently killed by undetermined factors, and early instars (first and second) too young to have had an opportunity to be parasitized. Parasitism rates of EAB larvae by the native North American hymenopterans (as a group) were calculated in the same way except that early instars (first and second) of EAB larvae were not excluded because it is known that *P. sulcata* attacks first instar host larvae (LSB, JJD unpublished). Mortality rates caused by woodpeckers and undetermined factors were calculated as the proportion of dead (or preyed upon) EAB larvae relative to the total number of all live stages of observed EAB larvae and/or pupae.

Data Analyses

To evaluate the establishment of *S. galinae* following field releases, we used a generalized linear mixed model (GLMM) with a binominal distribution to compare the proportion of trees with one or more broods of *S. galinae* across years after the initial releases and between the release and control treatments, with study site as a random effect (SAS Institute Inc. 2023). Based on the year of first detection of *S. galinae* parasitism in the control plots, as well as the distance of the first trees with detection of *S. galinae* parasitism to the parasitoid release point within each study site, we also estimated the mean (±SE) rate of spread by *S. galinae* (km/yr) after its releases began in 2015 using ArcGIS Pro (ESRI 2022).

We used a linear mixed model to analyze *S. galinae* abundance (mean number of broods observed per m^2 of sampled phloem area) in relation to parasitoid-release treatment and year after the initial release, with study sites as a random effect (SAS Institute Inc. 2023). To assess the impact of *S. galinae* on the level of EAB mortality, we first calculated the parasitism rate for each release or control plot from each sample tree based on the number of EAB larvae associated with larvae, pupae, and/or adult stages of *S. galinae* as a proportion of total EAB larvae. Then we used GLMM, based on the binominal distribution, to analyze the relationship between rates of parasitism in both release and control plots and years of study (sampling), with the study site as a random effect. Following approaches outlined in Duan et al. (2014b), we also calculated the net EAB population growth rate (R_0) for life tables with and without parasitism from either *S. galinae* or all the parasitoids (including North American native species and the earlier introduced biocontrol agent *T. planipennisi*). For the life table analysis, we pooled data from both parasitoid release and nonrelease control plots for each study site because *S. galinae* had quickly spread from all the release plots to all the control plots after its last field releases in the fall of 2017.

We calculated the EAB density based on the total number of live EAB larvae (all instars), pupae, and adults (indicated by fresh exit holes) observed divided by the phloem area (Y) of each sampled tree. The phloem area of each sample tree was estimated based on its DBH (X) by averaging estimates of the linear ($Y = 0.38*X - 1.76$) and second-order polynomial ($Y = 0.024*X^2 - 0.307*X + 2.63$) models relating DBH to phloem area, as per McCullough and Siegert (2007). All statistical analyses were carried out with JMP PRO 17.01 statistical software (SAS Institute Inc. 2023). Mean (±SE) rates of parasitism by resident hymenopteran parasitoids, including the earlier introduced agent *T. planipennisi* and the native hymenopterans (primarily *Atanycolus* spp. and *P. sulcata*), and separately, the mortality due to woodpecker predation and undetermined factors were also

calculated for both the release and control plots across the different study sites for the study period.

Results

Recovery, Spread, and Abundance of *S. galinae*

Throughout the study, a total of 248 ash trees were debarked. The average DBH of sample trees between parasitoid release and control plots ranged from 9.28 to 12.86 cm (Table 2). There was significant variation in the average DBH of sample trees among study sites (F = 2.82; df = 5, 237; P = 0.0169) and sampling year (F = 3.48; df = 4, 237; P = 0.0087), as well as between the parasitoid release and control plots (F = 16.24, df = 1, 237; P < 0.001). Parasitism of EAB larvae by *S. galinae* was observed as early as 2015 in one of 6 release plots. In both the release and control plots, the percentage of sampled trees with *S. galinae* parasitism increased sharply from 4.2%–8.3% in 2016 (1 yr after its initial releases) to 21.7–51.7% in 2017 when the last parasitoid releases were completed (Fig. 2). By 2020, the percentage of the sampled ash trees with *S. galinae* parasitism increased to the highest levels in both release (70.8%) and control (74.2%) plots (Fig. 2). The proportion of sampled trees with *S. galinae* parasitism significantly increased with year since initial release (F =12.23, df = 4, 50, P < 0.0001), but it was not significantly different between the parasitoid-release and nonrelease control plots (F = 2.366, df = 1, 50, P = 0.1302), (Fig. 3). The first detection of *S. galinae* parasitism in a control plot occurred at the Rose Lake site in the first year of sampling (spring of 2016), after the initial releases in the summer of 2015, approximately 3.6 km away from the nearest release point in the corresponding release plot at the same study site (Fig. 3). Based on the year of first detection of *S. galinae* parasitism on trees in each control plot and the distances of those trees to the parasitoid release point within each study site, a conservative estimate of the average rate of spread by *S. galinae* was determined to be 3.74 (±1.9 SE) km per year since its initial release in 2015.

Table 2. Mean (±SE) ash tree DBH, emerald ash borer larval density, percentage mortality caused by woodpeckers, undetermined factors (e.g., host tree resistance, undetermined diseases, competition, and/or weather), native parasitoids, and *T. planipennisi* in *Spathius galinae* release and nonrelease control plots across 6 study sites in Michigan from 2015 to 2020

Year of study[a]	Treatment plots	No. trees sampled	DBH (cm) of sampled trees	EAB density (n)/m² phloem	% EAB killed by woodpeckers	% EAB killed by undetermined factors	% EAB killed by native parasitoids	% EAB killed by *T. planipennisi*
2015	Release	23	11.01 ± 0.56	7.42 ± 1.40	22.0 ± 3.5	36.9 ± 6.6	9.9 ± 2.6	20.1 ± 7.5
	Control	27	9.90 ± 0.54	4.40 ± 1.08	13.1 ± 3.1	37.7 ± 6.8	22.3 ± 8.9	29.6 ± 7.5
2016	Release	23	10.73 ± 0.64	10.37 ± 1.86	50.1 ± 3.4	9.6 ± 1.2	16.8 ± 6.2	8.4 ± 1.9
	Control	25	9.28 ± 0.37	13.63 ± 1.85	42.4 ± 3.9	7.8 ± 1.6	5.2 ± 1.8	12.1 ± 5.0
2017	Release	25	12.86 ± 0.71	24.75 ± 3.28	40.0 ± 4.4	4.2 ± 0.8	9.9 ± 1.8	5.2 ± 1.2
	Control	26	10.77 ± 0.48	20.73 ± 2.57	36.4 ± 3.7	4.2 ± 0.9	5.5 ± 2.9	13. 4 ± 2.7
2018	Release	24	11.81 ± 0.54	4.62 ± 0.72	67.6 ± 3.1	11.6 ± 1.9	9.9 ± 2.4	6.6 ± 2.2
	Control	24	9.89 ± 0.33	6.11 ± 0.95	64.3 ± 3.4	12.4 ± 1.6	11.1 ± 1.9	10.0 ± 3.7
2020	Release	27	11.54 ± 0.69	2.77 ± 0.60	67.9 ± 3.8	5.9 ± 1.8	9.0 ± 3.6	5.7 ± 3.1
	Control	24	11.30 ± 0.51	4.40 ± 0.70	62.8 ± 3.2	6.5 ± 1.4	1.6 ± 0.7	15.1 ± 5.5

[a]Data were collected in early spring from 2016 to 2021, measuring parasitoid populations that developed the prior year (i.e., study year 2015–2020). Sampling was not carried out in the spring of 2020 (to measure the 2019 parasitism) due to the COVID-19 pandemic.

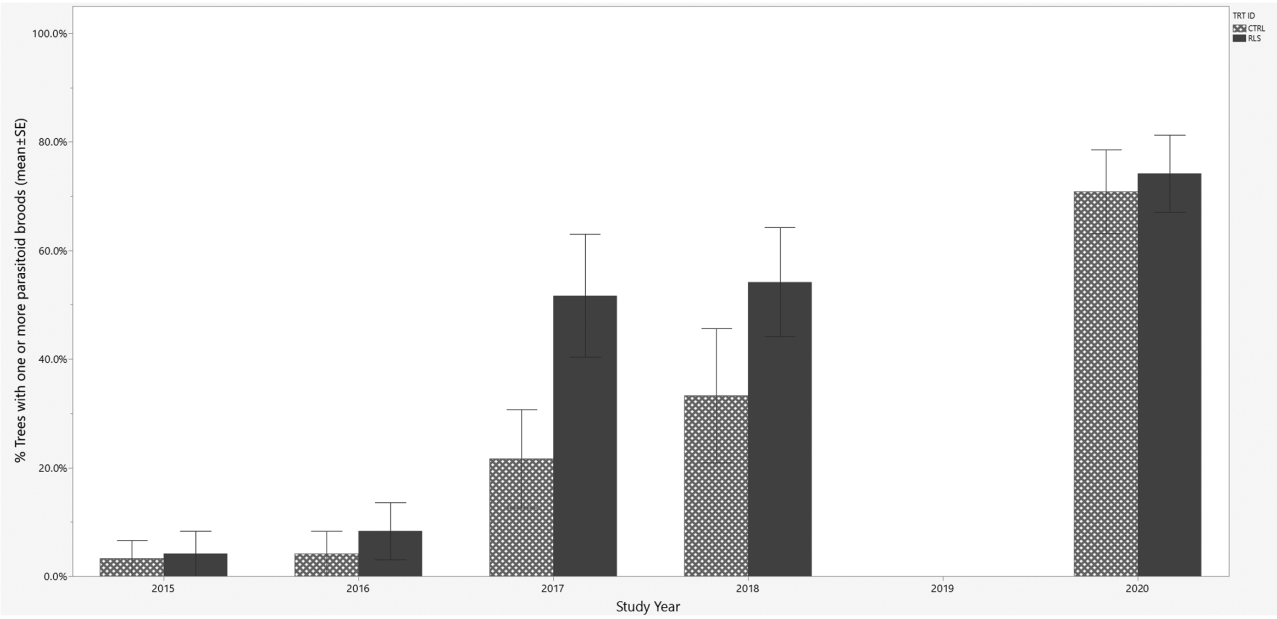

Fig. 2. Percentage (±SE) of sampled ash trees with ≥1 brood of *S. galinae* in release (RLS) and control (CTRL) plots across 6 study sites after releases in summer and fall 2015 to 2017. Data were collected annually in early spring from 2016 to 2021, measuring parasitoid populations that developed the prior year (2015–2020). Sampling was not carried out in the spring of 2020 (to measure the 2019 parasitism) due to the COVID-19 pandemic.

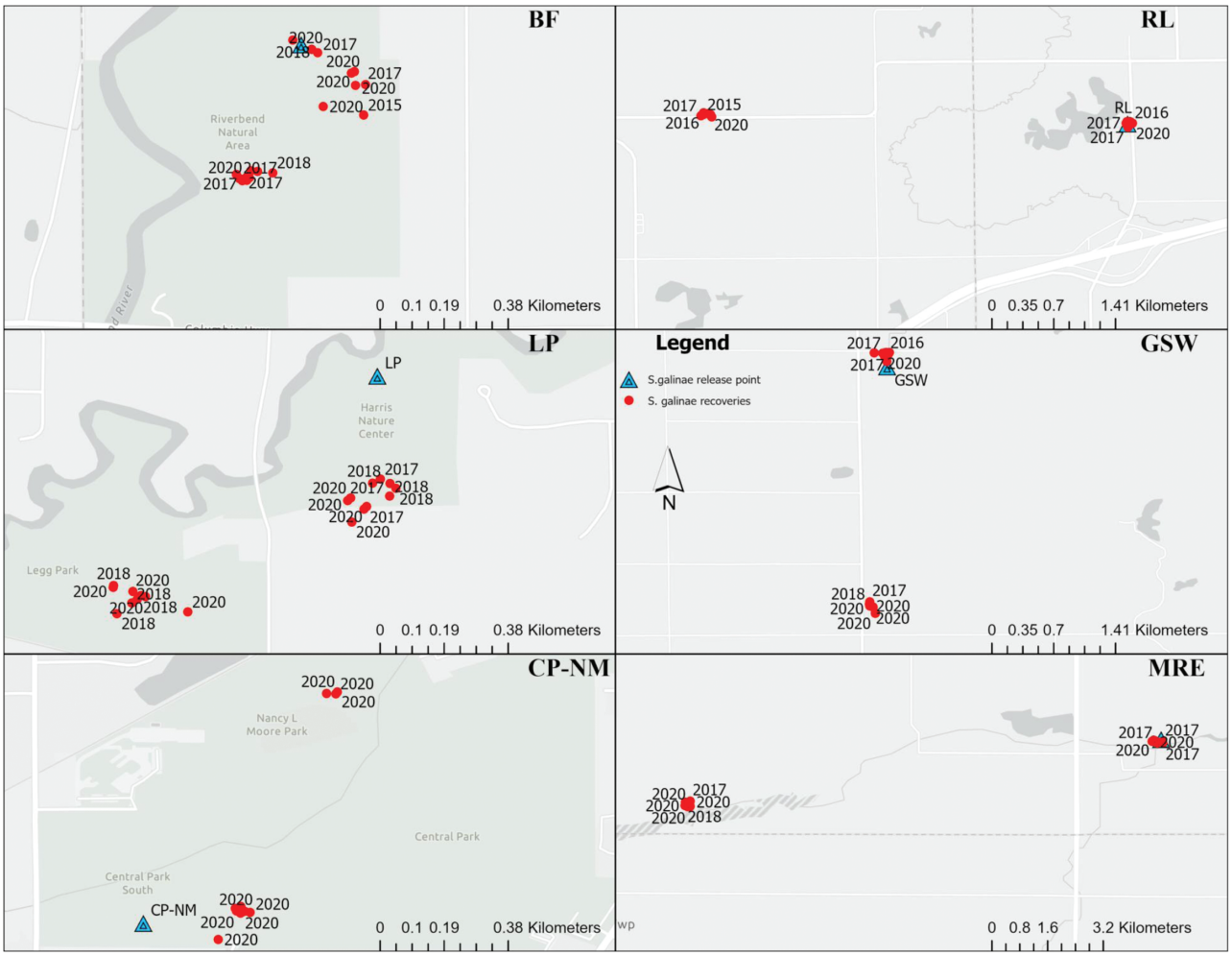

Fig. 3. Maps showing spatial distribution of trees with *S. galinae* parasitism in both release and control plots for each study site after releases from the summer of 2015 to 2017. BF = Williams F. Burchfield Park; CP-NM = Central Park and Nancy Moore Park; LP = Legg Park and Harris Nature Center; GSW = Gratiot-Saginaw State Game Area; MRE = Maple River State Game Area; RL = Rose Lake State Wildlife Area. Data were collected annually in early spring from 2016 to 2021, measuring parasitoid populations that developed the prior year (2015–2020). Sampling was not carried out in the spring of 2020 (to measure the 2019 parasitism) due to the COVID-19 pandemic.

A similar pattern of increasing abundance of *S. galinae* (number of broods/m^2 of sampled phloem area) was observed in both release and control plots over the study period (Fig. 4). The mean number of *S. galinae* broods within sampled trees increased from less than 1 brood per m^2 phloem area before 2017 to 2.53–2.57 broods per m^2 (for both release and control plots) in 2020, 3 yr after the last parasitoid releases. The mean *S. galinae* brood density significantly increased with the year since parasitoid releases (F = 26.78, df = 1, 238; P < 0.0001), but there was no significant difference between the release and control plots (F = 0.60, df = 1, 238; P = 0.4450).

Rates of EAB larval parasitism by *S. galinae* also increased in a similar pattern in both the parasitoid release and control plots following parasitoid releases (Fig. 5). In both parasitoid release and control plots, average rates of *S. galinae* parasitism across 6 study sites increased sharply from 4.3–6.7% in 2017 to 42.8–60.3% in 2020. While there were no significant differences in parasitism rates of EAB larvae by *S. galinae* between the release and control plots (F = 0.1241, df = 1, 50; P = 0.7263), the year since parasitoid release had a highly significant effect on *S. galinae* parasitism rate (F = 169.5; df = 1, 50; P < 0.001).

EAB Larval Mortality Caused by Other Factors

Across 6 different study sites, we observed similar levels of EAB larval mortalities between release and control plots due to factors other than *S. galinae* (Table 2). These factors included woodpecker predation, undetermined factors (including disease, putative host tree resistance, intraspecific competition, and/or weather), and parasitism by native North American parasitoids, and the previously established biocontrol agent *T. planipennisi*. Woodpeckers removed 13.1–67.9% of various immature stages (third instars to pupae) during the study period in both the parasitoid-release and control plots, while undetermined factors killed 4.2–37.7% of various immature stages (first instars to pupae). Native North American parasitoids (as a group), consisting primarily of *Atanycolus* spp. and *P. sulcata*, parasitized 1.6–22.3% of various EAB larvae (first to fourth instars) in parasitoid release and control plots across the different sites, while the earlier introduced agent *T. planipennisi* parasitized 5.7–29.6% of EAB larvae (third to fourth instars) (Table 2).

EAB Density and Net Population Growth Rates as Impacted by *S. galinae*

Mean EAB larval density (number of all live larval stages per m^2 of phloem) from both release and control plots increased from

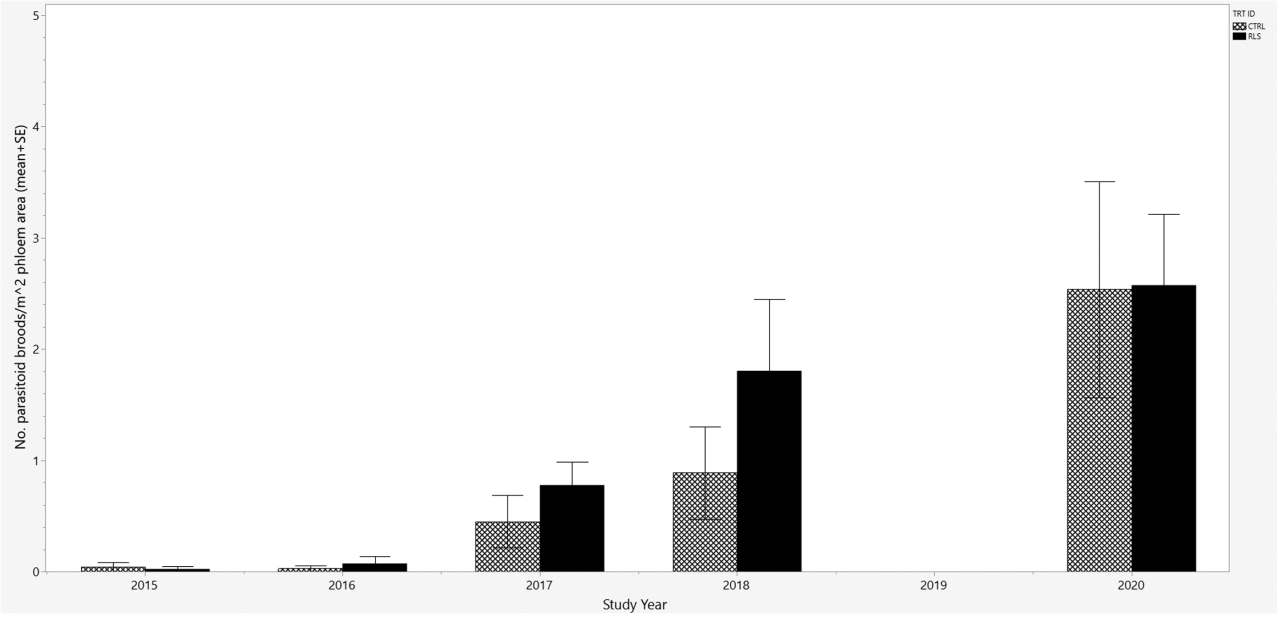

Fig. 4. Mean (±SE) number of *S. galinae* broods observed per sampled ash tree in both release (RLS) and control (CTRL) plots across 6 study sites after release from the summer of 2015 to 2017. Data were collected annually in early spring from 2016 to 2021, measuring parasitoid populations that developed the prior year (2015–2020). Sampling was not carried out in the spring of 2020 (to measure the 2019 parasitism) due to the COVID-19 pandemic.

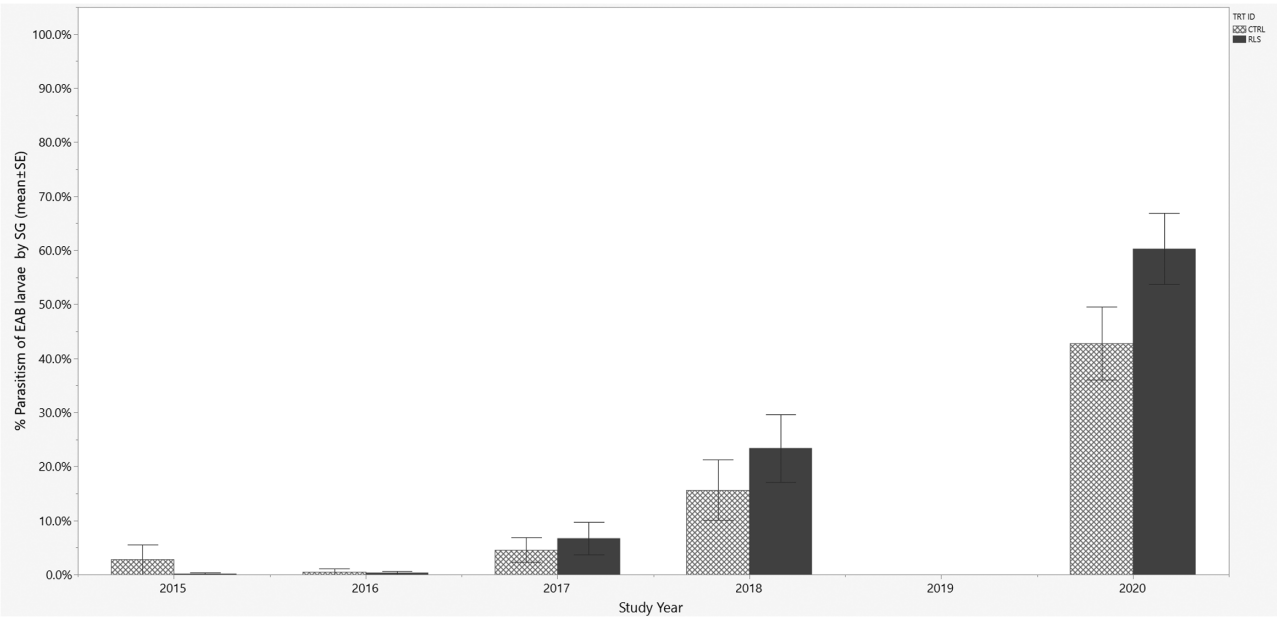

Fig. 5. Percent parasitism (±SE) of emerald ash borer (EAB) larvae by *S. galinae* (SG) in both release (RLS) and control plots (CTRL) at each of 6 study after releases from the summer of 2015 to 2017. Data were collected annually in early spring from 2016 to 2021, measuring emerald ash borer populations that developed the prior year (2015–2020). Sampling was not carried out in the spring of 2020 (to measure the 2019 parasitism) due to the COVID-19 pandemic.

4.40–7.72 in 2015 to its peak level of 20.34–24.75 in 2017, and then declined to its lowest level, 2.77–4.46 in 2020 (Table 2). While there was no significant difference in mean larval densities between the parasitoid release and control plots (F = 0.8402, df = 1, 230; P = 0.3603), there was a significant effect of time or study year on mean larval densities (F = 11.23, df = 4, 230; P < 0.0001), indicating highly significant temporal changes in EAB densities over the study period and following the establishment and spread of *S. galinae*.

Analyses of life tables constructed for each complete generation with all sources of mortality observed for each year at each of 6 study sites showed that the mean net population rate of increase (R_0) across sites was 1.61 (±0.13 SE) in 2015 (Fig. 6 – solid black line), indicating an expanding EAB population when the initial *S. galinae* releases at these sites began. The R_0 value increased to 2.40 (±0.18) in 2017, when the last parasitoid releases were completed (Fig. 6). After 2017, R_0 values declined sharply, by 67%, to 0.80 (±0.07) in 2018 and declined to the lowest level observed, 0.69 (±0.11), in

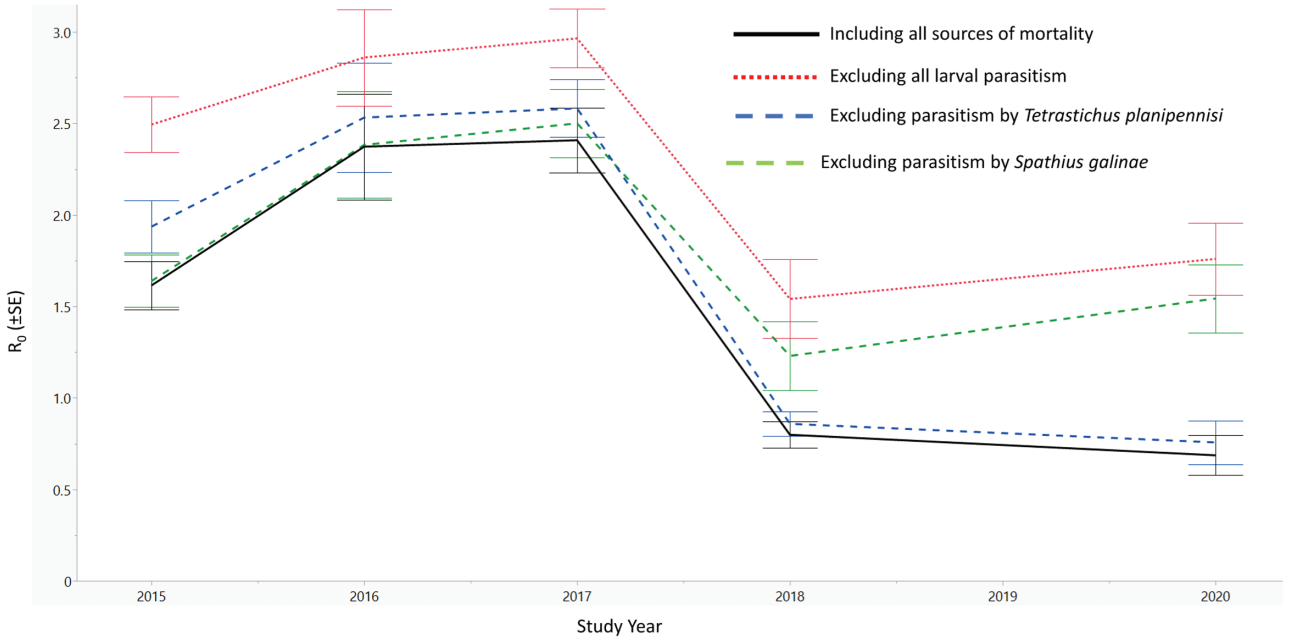

Fig. 6. Net population growth rates (R_0) of emerald ash borer (EAB) populations estimated from life table analyses for each observed generation across different study sites in Michigan after releases of *S. galinae* from the summer of 2015 to 2017. Life table analysis was based on data collected annually in early spring from 2016 to 2021, measuring parasitoid and emerald ash borer populations that developed the prior year (2015–2020). Sampling was not carried out in the spring of 2020 (to measure the 2019 parasitism) due to the COVID-19 pandemic.

2020, indicating a sharp reduction in EAB population densities from 2018 to 2020.

When parasitism by *S. galinae* was removed from the life tables constructed from the data, including all sources of mortality, changes to R_0 values were negligible from 2015 to 2017 during the initial period of *S. galinae* establishment (Fig. 6 – green dashed line). However, when *S. galinae* parasitism was removed from the life tables for 2018 through 2020, R_0 values were strongly affected, increasing approximately 54% in 2018 (from 0.80 with *S. galinae* to 1.23 without *S. galinae*) and 123% in 2020 (from 0.69 to 1.54). R_0 values were 35–55% lower when *S. galinae* parasitism was included than when it was removed from life table analyses during the period from 2018 to 2020 when parasitism rates had increased to high levels. Life table analyses also showed that the other parasitoids, including the previously introduced biocontrol agent *T. planipennisi* and several native North American parasitoids, also contributed to the reduction of R_0 values throughout the study (Fig. 6 – red and blue dashed lines). With the presence of all sources of mortalities in the EAB life tables, regression analyses revealed highly significant effects of time or study year ($F = 42.73$, df = 1, 45, $P < 0.001$) and parasitism by *S. galinae* ($F = 9.26$, df = 1, 45, $P < 0.005$) on pest population growth rates, as well as significant interaction between parasitism by *S. galinae* and time (year) following parasitoid releases ($F = 3.06$. df = 4, 45; $P < 0.05$).

Discussion

Following field releases during 2015–2017, *S. galinae* quickly established and spread to all control plots, which were 1.0–12.5 km from the nearest release plot within a study site. In addition, the proportion of sampled trees with *S. galinae* parasitism, parasitoid density, and parasitism rate all increased sharply in both the release and control plots after the last field releases were completed in 2017, reaching the highest observed levels in 2020. Life table analysis revealed that *S. galinae* alone caused a 35–55% reduction in the net population growth rate of EAB from 2018 to 2020. These results strongly indicate that *S. galinae* has firmly established and is now playing a significant role in reducing EAB populations in the area.

During our study, we conservatively estimated that *S. galinae* spread at an average rate of 3.7 (±1.9 SE) per year. Previous studies in Connecticut, Maryland, and New York have also documented the rapid spread of *S. galinae* at comparable or faster rates (Rutledge et al. 2021, Aker et al. 2022, Quinn et al. 2022). These results suggest that field releases of *S. galinae* can be spaced several kilometers apart without compromising its establishment and efficacy in controlling EAB populations. This could greatly expand and accelerate areawide-scale biocontrol programs by spreading release sites over broader geographic areas.

Spathius galinae has a long ovipositor (4–5 mm) and can attack emerald ash larvae in trees as large as 30 cm DBH (Murphy et al. 2017). In contrast, the previously-introduced larval parasitoid *T. planipennisi* has a much shorter ovipositor (1.5–2.5 cm) and is ineffective in larger ash trees with diameter at breast height (DBH) > 12.0 cm (Abell et al. 2012). Throughout our study, the mean DBH of sampled trees ranged from 9.3 to 12.9 cm, within the size-class range (pole-size) of infested ash trees susceptible to EAB parasitism by both *S. galinae* and *T. planipennisi*. However, EAB larval parasitism rates by *T. planipennisi* were much lower compared to *S. galinae* from 2018 and 2020 in both release and control plots. Declining *T. planipennisi* parasitism rates in sample trees across 6 study sites was most likely due to host resource partitioning based on the size or diameter of sampled ash trees rather than direct interspecific competition with *S. galinae* because both species have a strong ability to discriminate parasitized versus nonparasitized EAB larvae (Yang et al. 2012, Wang et al. 2015, Duan et al. 2017, 2021).

Our findings suggest that *S. galinae* will enhance the EAB biological control program in southern Michigan and similar regions, given its establishment success, high dispersal rate, and high impacts on EAB populations. Currently, *S. galinae* have been released in many other northern or mid-Atlantic US states (Mapbiocontrol 2023) as

well as in 3 Canadian provinces (Butler et al. 2022). More long-term studies are needed to determine if *S. galinae* has successfully established self-sustaining populations in additional regions where released, and to see how widespread its impact is on the reduction of EAB densities.

Besides parasitism by *S. galinae*, EAB larvae also suffered significant mortality from woodpecker predation, undetermined factors, and parasitism by native North American species as well as *T. planipennisi*, which was previously introduced. Among these factors, woodpecker predation accounted for the highest mortality, killing 13.1–67.9% of immature EAB stages across study sites and years of our study. Similarly, downy (*Dryobates pubescens* L.), hairy (*Leuconotopicus villosus* L.), and red-bellied (*Melanerpescarolinus* L.) woodpeckers have been found to be important natural enemies of EAB (Lindell et al. 2008, Koenig et al. 2013) but have highly variable predation levels among sites and between trees that depend on density of EAB larvae within trees and certain site characteristics (Lindell et al. 2008, Jennings et al. 2013). Mortality from native parasitoids was also significant at our study sites and ranged from 1.6 to 22.3% across sites and years. *Phasgonophora sulcata* Westwood and *Atanycolus* spp. Foerster are the most common native parasitoids attacking EAB with parasitism rates up to 40.7% for *P. sulcata* (Lyons 2015) and up to 71% for *Atanycolus cappaerti* Marsh and Strazanac (Cappaert and McCullough 2009). A recent study found that movement of ash logs containing native parasitoids to sites newly infested by EAB, augmented parasitism rates by *P. sulcata* and *Atanycolus* spp. over 3 yr and increased EAB mortality (Gaudon and Smith 2020). Host defense responses also contribute significantly to mortality of early-instar larvae (Duan et al. 2014b) and contributed to mortality from undetermined factors that ranged from 4.2 to 37.7% across our study sites. Efforts to improve host resistance through breeding programs are underway and will contribute to integrated management of EAB and ash restoration (Koch et al. 2012, 2015). How these other mortality factors interact with *S. galinae* in suppressing EAB populations is worthy of future research (e.g., Murphy et al. 2018).

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