Spread and phenology of *Spathius galinae* and *Tetrastichus planipennisi*, recently introduced for biocontrol of emerald ash borer (Coleoptera: Buprestidae) in the northeastern United States

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**HIGHLIGHTS**

- Emerald ash borer is responsible for catastrophic decline of ash in the U.S.
- Two larval parasitoids were introduced to control emerald ash borer 2015 – 2017.
- Post-release monitoring for phenology and spatial spread occurred in 2020 via sentinel logs.
- Both parasitoids were found throughout the sampled area and up to 14 km away from the release site.
- Phenologies of host and parasitoids in introduced area align.

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**ABSTRACT**

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive wood-boring pest of ash trees (*Fraxinus* spp.) in the United States. It is responsible for catastrophic decline of ash in urban and forested ecosystems, resulting in millions of dollars in economic losses. Biological control is one of the most promising management options available to reduce *A. planipennis* spread and impact. From 2015 to 2017, two larval parasitoids from the native range of *A. planipennis*, *Spathius galinae* Belokobylskij & Strazenac (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), were released in forested areas in New York and Connecticut. The purpose of this study was to measure the spread and seasonal phenology of these introduced parasitoids. From May to September 2020, sentinel ash logs containing EAB larvae were deployed in naturally occurring *A. planipennis* infested trees at each release site and at 2 km intervals up to 14 km away from the release site. Logs were replaced every two weeks and the parasitization rate was recorded. Each month, three trees from each release area were also cut and debarked to record *A. planipennis* infestation levels, natural parasitization rates, and *A. planipennis* and parasitoid phenology. We observed that both *S. galinae* and *T. planipennisi* emerged from the logs first deployed from the end of May to early June. Parasitization peaked in late July and mid-August, with sentinel logs deployed at each distance producing both species throughout the summer until mid-September. Both *S. galinae* and *T. planipennisi* were detected 14 km away from the release sites, the greatest distance away from the release sites sampled. Debarked trees produced similar seasonal patterns of parasitism. Our results indicate that the classical biological control program initiated several years ago has successfully produced a self-sustaining population of both *S. galinae* and *T. planipennisi*, which have been spreading widely and attacking the borer throughout the growing season. These findings strongly suggest that future release efforts may allow for wider temporal release windows and larger strategic spacing of release points across geographic regions.
1. Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is among the most destructive invasive tree pests in North America. Originally from Asia, it has spread throughout much of the northeastern and north central U.S. (Herms and McCullough, 2014). Emerald ash borer larvae feed within the phloem of ash trees (*Fraxinus* spp.), resulting in the catastrophic decline of ash in urban and forested ecosystems (Herms and McCullough, 2014). Costs of treatment and removal of infested ash trees in the northeastern United States over a 10-year period alone have been projected to exceed over $25 billion (Kovacs et al., 2010).

In response to the lack of practical treatments for emerald ash borer infestations in natural forests, classical biological control has been pursued as a long-term, low-cost, low-risk, self-sustaining management option for reducing emerald ash borer injury and spread on a landscape scale (Duan et al., 2018). Several parasitoids have been selected for ongoing release efforts, including two larval parasitoids: *Spathius galinae* Belokobylskij & Strazenac (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae). Releases of these parasitoids occurred in New York state and Connecticut from 2015 through 2017 where both species have since established (Abell et al., 2014; Duan et al., 2019; Jennings et al., 2018, 2016; Jones et al., 2019). In laboratory flight-mill studies, *T. planipennisi* has been observed flying as far as 1.81 km in a 24-hour period (Fahrner et al., 2015), but its dispersal ability in the field is unknown. No such studies of *S. galinae* have been conducted. Although these species have been successfully introduced as biocontrol agents against EAB, little is known about their dispersal and ecology in both their native range and their newly introduced environment.

Monitoring the establishment and spread of introduced biological control agents is essential to determining program success. To this end, effective sampling methods must be developed. While there are several methods available with which to monitor EAB larval parasitoids, two methods surpass others in efficacy: bark peeling of trees infested with EAB and deployment of sentinel logs containing suitable stages of host larvae (Rutledge et al. 2021). These methods allow for the documentation of parasitoid geographic range and seasonal activity. Using these sampling methods, here we determine the spatial distribution relative to release points and the phenology of *T. planipennisi* and *S. galinae* in two Northeast States, where these two biocontrol agents were released 3–5 years earlier.

2. Materials and methods

2.1. Study sites

All studies occurred in two locations: Dutchess County, NY (42°0′15.60″N, 73°54′30.91″W) and Middlesex County, CT (41°35′24.26″W, 72°39′43.79″W). Releases of *T. planipennisi* and *S. galinae* occurred in these locations from 2015 to 2017 (Table 1). Study sites consisted of mixed hardwood forests containing ash (*Fraxinus* spp.) showing signs of EAB infestation (e.g. reduced crown condition, epicormic shoots, and adult EAB exit holes in bark). Sentinel logs were used to study the emergence phenology and spatial spread of the two parasitoids. In addition, at each release location, trees were cut and debarked to record the occurrence and phenology (stage) of EAB larvae and associated larval parasitoids. Details of these methods are described below.

Table 1

<table>
<thead>
<tr>
<th>States</th>
<th>Site codes</th>
<th>Year</th>
<th>Tetrastichus planipennisi</th>
<th>Spathius galinae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connecticut</td>
<td>CT3</td>
<td>2015</td>
<td>16,800</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2016</td>
<td>1909</td>
<td>903</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2017</td>
<td>–</td>
<td>98</td>
</tr>
<tr>
<td>New York</td>
<td>NY1</td>
<td>2015</td>
<td>4300</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2016</td>
<td>1918</td>
<td>903</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2017</td>
<td>–</td>
<td>98</td>
</tr>
</tbody>
</table>

2.2. Parasitoid spread

Sampling occurred from May 28th through September 15th, 2020. At each site, two transects were created, sharing a release site as their origin (Fig. 1). Within these transects, sentinel log sampling occurred at the release site and at 2 km intervals leading away from the release site up to 14 km away, with one log deployed per sampling location. Sentinel logs were created using a protocol similar to that described in Abell et al. (2015), Abell et al. (2016) either in the USDA-APHIS facility in Brighton, MI or in USDA-ARS BIIRU in Newark, DE. Videos demonstrating the sentinel log production process can be viewed at: https://www.youtube.com/channel/UCGvVm2jzhMrU4-DGjvD5Q. All emerald ash borer larvae used to produce sentinel logs were reared in the laboratory under standard rearing conditions as described in Duan et al. (2014). Healthy 3rd or 4th instar larvae were dissected out of the rearing ash logs and immediately inserted back to freshly cut green (*Fraxinus pennsylvanica* or white (*F. americana*)) ash logs. For larval insertion, five thin bark-flaps were created along the longitudinal axis of each log using a razor-sharp utility knife. Under each bark flap, a narrow channel (approximately 2 mm deep by 3–4 mm wide by 30 mm long) was fashioned as an artificial gallery (or groove) in the exposed cambium and sap wood using a #11 palm-handled wood veneer (Woodcraft Supply LLC, Parkersburg, WV, USA). Each EAB larva was then inserted with soft forceps into a groove with larval head toward flap hinge and spiracles facing out. The bark-flap was then secured closed using thin bands of Parafilm. For field deployment, the base of the log was placed into a specimen cup filled with rockwool cubes (Grodan, Milton, ON, Canada) and filled with water, then wrapped in paper towels and covered with paraffin. A wire harness was constructed around the cup to hold the log in place. Logs were affixed at breast height to ash (*F. pennsylvanica* or *F. americana*) trees showing signs of emerald ash borer infestation. Sentinel logs remained in the field for two weeks, after which they were retrieved, and a new sentinel log was deployed in its place.

Retrieved logs were held in a shaded outdoor insectary for eight weeks in clear plastic tubes (6.3 cm diameter, 21.6 cm long) streaked with honey to contain and sustain any emerging parasitoids. Logs were check for emergence weekly, and all emerging parasitoids were counted and identified. After eight weeks, the logs were dissected. The fate and developmental stages of all emerald ash borer larvae and parasitoids were recorded.

2.3. Phenology

Tree debarking data were collected as described in Jennings et al. (2016). Pole-size trees were selected for sampling (mean DBH = 9.7 ± 0.1 cm). Three live trees with visible signs of EAB infestation (e.g. epicormic shoots, exit holes, crown decline), per release site were selected for destructive sampling once per month from June through August. Trees were felled and bark was removed from the entirety of the tree. All EAB larvae and parasitoids were collected and their fates were recorded. Upon dissection, unemerged parasitoids were identified to the respective species based on the characteristics of the brood — e.g., size and presence or absence of cocoons (Duan et al. 2011, Duan et al., 2014).

While previous field studies in the study area had reported the presence of native parasitoids (Duan et al. 2019, 2020), none were detected during this study.
2.4. Analysis

All data were analyzed using SAS JMP Pro 15.1.0 (SAS Corporation, Cary, NC). For the tree peeling data, the proportion of all L3–L4 larvae parasitized by each parasitoid by site over time were analyzed via likelihood ratio test based on generalized linear (binomial logit link) model, with debarking date as the main effect. Sentinel log data were analyzed similarly, with the proportion of viable larvae parasitized by each site and transect over time and distance was analyzed via the same method, with time and distance as the main effects. Larvae were deemed viable if there was visible evidence of feeding after insertion.

3. Results

3.1. Parasitoid spread

Both *T. planipennisi* and *S. galinae* were recovered from sentinel logs at all transect distances, including up to 14 km away from the release point (Figs. 2, 3). In the CT transects, there were no significant differences in the proportion of larvae attacked by *T. planipennisi* in the northern transect ($\chi^2 = 0.24$, DF = 1, $P = 0.62$) (Fig. 2A), but there was a significant difference in parasitization by distance from the release point in the southern transect ($\chi^2 = 9.39$, DF = 1, $P = 0.0022$), with relatively greater levels of parasitism observed farther from the release point (Fig. 2B). *Spathius galinae* parasitization also did not vary by distance from the release point in CT in the northern ($\chi^2 = 1.02$, DF = 1, $P = 0.31$) (Fig. 3A) or southern ($\chi^2 = 3.79$, DF = 1, $P = 0.052$) (Fig. 3B) transect. Results were similar in NY, with no significant differences in parasitization by distance in: *T. planipennisi* in the eastern transect ($\chi^2 = 0.0050$, DF = 1, $P = 0.94$) (Fig. 3C), *T. planipennisi* in the southern transect ($\chi^2 = 0.0035$, DF = 1, $P = 0.95$) (Fig. 2D), or *S. galinae* in the southern transect ($\chi^2 = 1.71$, DF = 1, $P = 0.19$) (Fig. 3D). However, there was a statistically significant relationship between distance from the release point and *S. galinae* parasitization in the eastern NY transect ($\chi^2 = 4.24$, DF = 1, $P = 0.04$) (Fig. 3C), with the mean proportion of larvae parasitized by *S. galinae* being greatest farthest from the release point.

3.2. Phenology

Both parasitoids attacked larvae in sentinel logs deployed throughout the season (June through September). Greatest levels of parasitization by *T. planipennisi* were observed in logs retrieved in mid to late July, with peak mean parasitization observed in the southern CT transect where 44.4 ± 1.0 % of viable larvae were parasitized. There was no significant difference in the rate of parasitization by *T. planipennisi* over time ($P > 0.05$) (Fig. 4), except in the case of the southern CT transect, where logs retrieved later in the season were parasitized at relatively lower rates ($\chi^2 = 8.26$, DF = 1, $P = 0.0041$) (Fig. 4B). *Spathius galinae* parasitization over time was similar, with mean overall parasitization peaking at up to 42.9 ± 1.1 %, remaining relatively constant throughout the season. There were no significant differences in the rate of parasitization of viable EAB larvae by *S. galinae* over time ($P > 0.05$) (Fig. 5) other than in the eastern NY transect ($\chi^2 = 5.2$, DF = 1, $P = 0.0023$) (Fig. 5B). Total parasitoid emergence was similar in both states, with peak parasitization observed in logs retrieved at the end of July (Fig. 6).

A total of 867 EAB were recorded from harvested and debarked trees across both sites. Of these, 37.3% were live larvae, 3.6% were dead larvae, 21.7% were undetermined, 11.8% were larvae removed by woodpeckers, 5.0% were exit holes from emerging adults, and 20.7% were parasitized larvae. 51.2 % of the larvae collected were stages L3–L4 and therefore vulnerable to parasitization. Parasitoids were detected in the first samples collected on 5/14/20 in CT and 5/28/20 in NY and in the final sampling date in both locations on 10/5/20. In CT, sample date did not significantly affect the proportion of L3–L4 EAB larvae parasitized by *T. planipennisi* ($\chi^2 = 0.247$, DF = 1, $P = 0.619$) (Fig. 7A) or *S. galinae* ($\chi^2 = 1.97$, DF = 1, $P = 0.1601$) (Fig. 8A). In NY, date also did not significantly affect the proportion of EAB larvae parasitized by *T. planipennisi* ($\chi^2 = 0.122$, DF = 1, $P = 0.727$) (Fig. 7B). However, the date of sampling significantly affected *S. galinae* in NY ($\chi^2 = 4.27$, DF = 1, $P = 0.0388$), with significantly greater levels of parasitization observed in July than in the other months (Fig. 8B).

4. Discussion

Both *T. planipennisi* and *S. galinae* have established abundant populations three years after the most recent release and spread at least 14 km away from their initial release sites in both sites. Other studies have also observed strong dispersal of *T. planipennisi*. Jones et al. (2019) caught *T. planipennisi* in yellow pan traps deployed along the entirety of a 20 km transect in central New York three years after the most recent release in the area sampled. In Michigan, *T. planipennisi* was found have spread up to 3 km away from the release site each year (Duan et al., 2013). While it has been speculated that *S. galinae* may be able to spread more quickly than *T. planipennisi* due to its larger body size (Duan et al., 2019), our study suggests that both species have spread quickly in the regions in which they have been introduced. The continued spread and establishment of *T. planipennisi* and *S. galinae* is likely dependent upon the continued availability EAB for parasitization in the landscape (French and Travis, 2001). Decreasing EAB density may negatively impact EAB parasitoid persistence (Reeve, 1988), but long-term population dynamics are currently unknown. Future monitoring efforts in

![Fig. 1. Sampling locations in Connecticut (A) and New York (B). Sampling locations were selected by ash availability and location. In Connecticut, locations followed rivers, while in New York, sample sites followed roads. Parasitoid releases occurred for several years prior to sampling at the points in yellow. Sentinel logs were deployed at 2 km intervals leading away from the release site up to 14 km. One sentinel log containing five L3-L4 EAB larvae was deployed at each location at two-week intervals from May through September. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
different regions would be beneficial in determining region-specific effects on dispersal or spread, if any. Based on our findings, the monitoring radius several years after release should be expanded beyond 14 km once establishment is confirmed in order to better determine its dispersal abilities. Future monitoring efforts should continue to include tree debarking and ash inventories in addition to sentinel logs in order to determine baseline host availability for the sample area.

The phenology of both parasitoids was relatively synchronous between regions and study methods, both with each other and with known EAB phenology (Herms and McCullough, 2014). Studies in other regions have demonstrated a possible asynchrony in the phenology of EAB and S. galinae (Ragozzino et al., 2020), but that does not seem to be the case in the northeastern U.S., both according to our data and the findings of Jones et al. (2020). However, predictive modeling suggests that climate change may negatively affect T. planipennisi overwintering survival and subsequent establishment, particularly in the southern portions of its introduced range (Gould et al., 2020). Climate change may exacerbate asynchrony of emerald ash borer and its parasitoids. As such, long-term studies on EAB population dynamics and ash recovery are needed.

Additionally, life table analyses are needed to examine the cumulative proportion of larvae parasitized rather than the mean percentage of larvae parasitized.

Sentinel logs seem to detect T. planipennisi at higher rates than debarking, while for S. galinae the inverse is seen. Spathius galinae has a longer ovipositor than T. planipennisi (Abell et al., 2012; Murphy et al., 2017). Duan et al. (2017) found that in naturally occurring trees, S. galinae predominate in pole size trees, and T. planipennisi are more abundant in saplings. The sentinel logs were small in diameter and had relatively thin bark compared to the trees on which they were deployed. Perhaps the thickness of the bark of the tree on which the sentinel log bolt is deployed affects parasitoid foraging behavior. There may be additional differences in the foraging behavior of S. galinae and T. planipennisi that have yet to be determined. Future studies should examine the cues utilized by both species in locating hosts.

5. Conclusions

Together, our findings provide strong field evidence that the two...
Fig. 4. Mean proportion of L3–L4 EAB larvae parasitized by *T. planipennisi* by sentinel log retrieval date in the: A) northern CT transect, B) southern CT transect, C) eastern NY transect, and D) southern NY transect. Asterisks indicate a statistically significant relationship between distance and parasitism according to logistic regression (*P* < 0.05).

Fig. 5. Mean proportion of L3–L4 EAB larvae parasitized by *S. galinae* by sentinel log retrieval date in the: A) northern CT transect, B) southern CT transect, C) eastern NY transect, and D) southern NY transect. Asterisks indicate a statistically significant relationship between distance and parasitism according to logistic regression (*P* < 0.05).

Fig. 6. Total female *S. galinae* and *T. planipennisi* emergence from sentinel logs deployed in CT (A) and NY (B) by log retrieval date. Logs were deployed for two weeks, then retrieved and monitored for parasitoid emergence. It’s important to note that while both parasitoids are gregarious, *T. planipennisi* broods are much larger (30–70 per parasitized host) than those (9–15 per parasitized host) of *S. galinae* (Duan and Oppel 2012, Duan et al., 2014).
recently introduced biocontrol agents have established self-sustaining populations and spread quickly up to 14 m of radius away from the original release points. To our knowledge, our study provides the first evidence of *S. galinae* spread widely beyond a release site. Along with the moderate levels of EAB larval parasitism detected in both Connecticut and New York, these results bode well for sustained biological control of EAB. Future studies should continue to assess the long-term impact of *T. planipennisi* and *S. galinae* on EAB populations in Northern U.S., where both parasitoids have been released in the last three to five years.

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**References**


