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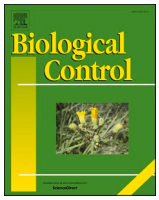

Factors affecting yellow pan trap captures of the emerald ash borer biocontrol agents *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae): Implications for monitoring establishment and seasonal abundance

Toby R. Petrice [a,b,*], Therese M. Poland [a,b], Leah S. Bauer [a,b], John S. Strazanac [c], Jian J. Duan [d], Jonathan M. Schmude [d], F. William Ravlin [b]

[a] USDA Forest Service, Northern Research Station, Lansing, MI 48910, USA
[b] Michigan State University, Department of Entomology, East Lansing, MI 48824, USA
[c] West Virginia University, Department of Plant and Soil Sciences, Morgantown, WV 26506, USA
[d] USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 19713, USA

HIGHLIGHTS

- The Asiatic parasitoids *Spathius galinae* and *Tetrastichus planipennisi* have been released in North America to manage emerald ash borer.
- Yellow pan trap captures of *Spathius galinae* and *Tetrastichus planipennisi* varied among sites and years, and occurred across a wide range of growing degree days.
- Placing yellow pan traps on trees with suppressed or intermediate crown classes, abundant epicormics sprouts and smaller diameters increased *T. planipennisi* capture rates.
- *Tetrastichus planipennisi* yellow pan trap captures had a positive relationship with parasitized EAB larval densities from the previous year.

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ABSTRACT

Yellow pan traps are frequently used to monitor establishment of hymenopteran parasitoids introduced for biological control of the invasive ash pest, emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). To evaluate and improve their efficacy as a monitoring tool, we conducted field studies during 2016–2019 in southern Michigan where the introduced hymenopteran larval parasitoids, *Tetrastichus planipennisi* Yang (Eulophidae) and *Spathius galinae* (Braconidae) Belokobylskij & Strazanac are established. Our objectives were to describe parasitoid seasonal abundance, identify host tree variables that might influence parasitoid capture numbers (i.e., number of adults captured) and capture rates (i.e., number of positive traps per collecting period), and compare parasitoid trap captures to parasitized EAB larval densities. Yellow pan trap capture numbers and rates of both species were low for most study sites and years. Captures of both parasitoid species occurred throughout the growing season and neither showed a consistent trend in seasonal abundance. Emerald ash borer infested trees with suppressed or intermediate crown classes had higher *T. planipennisi* capture numbers and rates than dominant or codominant crown classes. During some years, *T. planipennisi* capture rates increased with decreasing tree diameter at breast height (DBH) and *T. planipennisi* capture numbers increased with increasing numbers of epicormics sprouts on host trees. *Spathius galinae* captures had no significant relationship with host tree variables but low overall capture numbers, due to its more recent introduction, may have prevented detection of any relationships. *Tetrastichus planipennisi* adult capture numbers were positively related to previous year's densities of *T. planipennisi* parasitized larvae dissected from trees. *Spathius galinae* capture numbers were not correlated with parasitized EAB larval densities. We discuss the relevance of our findings to improve yellow pan trap efficacy for monitoring introduced EAB larval parasitoids.

* Corresponding author.
E-mail address: toby.petrice@usda.gov (T.R. Petrice).

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1. Introduction

The invasive pest of ash trees (*Fraxinus* spp.), emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), continues to spread throughout North America, threatening to functionally extirpate some native ash species (Haack et al., 2002; Herms and McCullough, 2014; Poland and McCullough, 2005; Ward et al. 2021). In efforts to manage EAB, releases of four Asiatic parasitoid species as EAB classical biological control agents in North America began in 2007 (Bauer et al., 2008, 2015; Duan et al., 2018a). These include an egg parasitoid, *Oobius agrili* Huang and Zhang (Encyrtidae), and three larval parasitoids, *Tetrastichus planipennisi* Yang (Eulophidae), *Spathius agrili* Yang (Braconidae) and *Spathius galinae* Belokobylskij and Strazanac (Braconidae). The two *Spathius* species are ectoparasitoids while *T. planipennisi* is an endoparasitoid.

Releases of *O. agrili*, *T. planipennisi*, and *S. agrili* began in Michigan in 2007, followed by releases of *S. galinae* in 2015 (Bauer et al., 2008; Bauer et al., 2015; Duan et al., 2018a). *Spathius agrili*, however, did not successfully establish in northern regions, despite numerous releases throughout the upper Midwest and northeastern U.S. Consequently, releases of *S. agrili* are now restricted to latitudes below 40°, where climatic conditions are more similar to where parasitoid source populations were collected (Bauer et al., 2015; Duan et al., 2018a, 2022).

Tetrastichus planipennisi, *S. galinae*, and *O. agrili* are now considered established in many areas of the Upper Midwest and Northeastern U.S., and in southeastern Canada (Bauer et al., 2015; Duan et al., 2019, 2021, 2022; MapBioControl 2022). Releases of these parasitoids continue in areas where EAB is established, including latitudes much farther south than their endemic ranges in Asia. As parasitoids are introduced into new areas, resource managers need practical methods to monitor their establishment, abundance and spread.

Yellow pan traps are regularly used to monitor EAB parasitoid establishment because of their low technological requirements, ease of deployment and collection, ability to capture all four introduced parasitoids, and avoidance of destructive host tree sampling methods (USDA APHIS/ARS/FS, 2021; Petrice et al., 2021; MapBioControl, 2022). For example, Jones et al. (2019) used yellow pan traps to monitor dispersal of *T. planipennisi* and *S. agrili*. In a study by Parisio et al. (2017), yellow pan traps had similar *T. planipennisi* recoveries as sentinel larval logs (consisting of small ash logs with EAB larvae inserted) and field-collected EAB-infested host logs that were reared in the laboratory for adult parasitoid emergence. In contrast, Rutledge et al. (2021) found that both sentinel larval logs and host tree dissections had significantly higher *T. planipennisi* and *S. galinae* compared to yellow pan traps. Although some methods may be more effective at detecting smaller parasitoid populations, available survey resources and goals must be considered when choosing a survey method.

It is likely that yellow pan trap captures of parasitoids are related to certain host tree variables, such as size, canopy condition, and EAB infestation levels. For example, *T. planipennisi* prefers small diameter trees and saplings, while *S. galinae* prefers pole-size and larger trees (Duan et al., 2021). In addition, Johnson et al. (2014) found that *S. agrili* was attracted to host plant cues such as ash stems containing EAB larvae and ash leaf tissue. Because parasitoids often use a combination of host and host-plant cues when locating their hosts (Vinson, 1998), more than one tree variable may be important for attracting introduced parasitoids to host trees. Therefore, yellow pan trap sampling efficacy may be improved by selecting trees that are most attractive to introduced EAB larval parasitoids.

Although *T. planipennisi* and *S. galinae* have been frequently recovered in yellow pan traps, the optimal seasonal timing for sampling these species has not been determined. Both species are gregarious multivoltine species that overwinter as prepupae or larvae within EAB larval galleries. *Tetrastichus planipennisi* produces an average of 57 progeny per female and requires ca. 546 GDD_{10} per generation [based on development time at 25 °C; (Duan et al., 2011, 2018b)]. *Spathius galinae* average 43 progeny per female and require ca. 543 GDD_{10} to complete one generation [based on development rate and pre-oviposition period at 25 °C; (Watt et al., 2016)]. Therefore, adult parasitoid abundance should increase with each consecutive generation as the season of parasitoid activity progresses. Furthermore, if parasitoid generations are synchronized, multiple peaks of adult abundance should occur throughout the season of activity. Knowing when adult parasitoids are most abundant would be highly valuable for determining trap deployment timing to improve efficacy and reduce sampling effort.

In efforts to improve introduced parasitoid captures in yellow pan traps, we collected EAB larval parasitoids in yellow pan traps attached to ash trees throughout the 2016–2019 growing seasons. For each ash tree, we recorded tree variables that we anticipated could affect larval parasitoid captures, including tree DBH (diameter at breast height), crown class, bark texture, and signs and symptoms of EAB attack. We also dissected trees and saplings at each site to estimate EAB parasitized larval densities. Our objectives were to: a) describe the relative seasonal occurrence of adult EAB larval parasitoids, b) identify tree-related variables that are related to yellow pan trap captures, and c) determine if yellow pan trap capture rates are correlated with parasitized EAB larval densities estimated from dissected trees. This information could improve parasitoid recovery and monitoring efficacy with yellow pan traps at parasitoid release sites.

2. Methods

2.1. Study sites and yellow pan traps

We conducted yellow pan trap sampling at three sites in south-central Michigan (Gratiot-Saginaw State Game Area, Harris Nature Center, and Legg Park) from 2016 to 2019. Detailed information on parasitoid release and establishment histories for study sites can be found in (Duan et al., 2013) and MapBioControl (2022), and a brief summary is presented in Table 1. In 2016, 40 yellow pan traps were placed on live ash trees irrespective of evidence of EAB infestation within a ca. 0.25-hectare area at each study site. In 2017–2019, due to tree mortality and the lack of trees with no signs of EAB infestation at some sites, only 10 yellow pan traps were deployed within each of the same 0.25-hectare areas, selecting only trees that displayed signs and symptoms of recent EAB attack (e.g., fresh woodpecker feeding holes, epicormics sprouts, and/or canopy dieback). Canopy decline of these trees varied from 0 to 90% and all trees had some live phloem present for EAB oviposition and larval development. Individual trees sampled varied from year to year depending on their condition and ranged from 5.0 to 30.3 cm diameter at breast height (DBH; 1.37 m above ground).

Yellow pan traps were constructed and deployed following slightly modified methods as described in EAB Biocontrol Guidelines (USDA APHIS/ARS/FS, 2021). We secured individual yellow plastic bowls (diam. = 18 cm, vol. = 355 mL; Nationwide Party, Brooklyn, NY, Item # 65015, color = Sunshine Yellow) to inverted lids of 7.5 cm diam. petri dishes with hot melt adhesive. Holes were drilled through the bottom of these units aligned with predrilled holes in metal shelf brackets (15-cm vertical length × 20-cm horizontal length). Small bolts and nuts were used to secure each bowl/petri dish unit to the shelf bracket. At the study sites we attached the units with wood screws through the shelf bracket to the south side of ash trees ca. 1.75 m above the ground. We nested a second yellow bowl inside the attached yellow bowl to serve as the trap collection bowl and allow easy removal of trap contents. A solution of 50% food-grade propylene glycol (ChemWorld.com, Taylor, MI) and 50% water was added to the nested bowl to kill and preserve insects trapped in the bowl. We added ca. 5 mL per L of unscented dish detergent to the solution to reduce surface tension.

We collected yellow pan trap samples once every two weeks in 2016 and once every week in 2017–2019. To collect samples, we poured trap contents through paint strainers to separate insects from the trapping solution, placed labelled paint strainers in resealable plastic bags, and

froze them for later processing. Samples were inspected for EAB parasitoids in the laboratory using a dissecting microscope. Suspect EAB larval parasitoids were removed and identifications confirmed using species descriptions and illustrations (Yang et al., 2006; Belokobylskij et al., 2012; USDA APHIS/ARS/FS, 2021).

For each tree sampled, we recorded the DBH; crown class [open grown/edge tree (at least 50% of entire tree receives full sunlight), dominant/codominant (tree crown at or above forest canopy level, but only tree canopy receives direct sunlight), or intermediate/suppressed (tree canopy below forest canopy level)]; bark texture (i.e., smooth = no or few furrows, intermediate = shallow furrows, or coarse = deep furrows); canopy dieback (10% increments estimated in late summer); number of epicormics sprouts growing from tree bole (up to 4 m above ground); and number of recent woodpecker feeding holes (up to 4 m above ground).

2.2. Tree dissections

At each site and in close proximity (ca. < 50 m) of the 0.25-hectare plot used for yellow pan trap sampling, we felled and dissected 4–5 ash trees (8.0–15.6 cm DBH) in fall 2015–2017, and early spring 2019 (these trees contained EAB larvae parasitized during the 2018 growing season). In addition, we felled and dissected 10 sapling-size ash trees (3.6–5.0 cm DBH) at each site in early spring 2019. Tree dissections to recover EAB larvae parasitized in 2019 were not conducted due to Covid-19 restrictions. We selected trees and saplings with fresh woodpecker feeding holes for dissections (Koenig and Liebhold, 2017). A draw knife was used to remove the bark from the entire stem and all branches that were > 2.5 cm in diameter. Phloem area for each tree or sapling was calculated using models described in McCullough and Siegert (2007). The number of EAB larvae attacked by each parasitoid species was recorded. Immature parasitoids were identified based on larval morphology and parasitoid biology. For example, *T. planipennisi* is a gregarious endoparasitoid that exits EAB larvae when mature and forms exarate pupae. *Spathius galinae* is a gregarious ectoparasitoid that spins silken cocoons within which they will later pupate (Duan et al., 2019). Undamaged immature parasitoids for each species were reared to adults in the laboratory to confirm identifications. The number of EAB larvae parasitized by each species was divided by the total ash phloem surface area dissected for each site and year to standardize parasitized EAB larval densities.

2.3. Data analysis

For each site, the number of EAB parasitoids of each species captured per week was expressed as a percentage of the total number captured throughout the season and plotted against the number of accumulated GDD_{10} (growing degree days base 10 °C, start date = Jan. 1). A base temperature of 10 °C was used because this is a common standard for monitoring insect development in the field (Herms 2004). GDD_{10} data were downloaded from the nearest weather station to each site (Michigan State University Enviroweather, 2019). Weather stations selected were within 10 km of each study site.

Tetrastichus planipennisi capture rates (i.e., proportion of traps that recovered at least one parasitoid) in 2016 were compared between trees with or without signs or symptoms of recent EAB attack (i.e., epicormics sprouts, recent woodpecker feeding holes, or canopy dieback) using logistic regression (PROC LOGISTICS; SAS Institute, 2012). *Spathius galinae* recoveries were not compared because only one individual was recovered in 2016. To determine if tree variables affected EAB parasitoid captures, we selected a series of 11 multi-location generalized linear mixed models (PROC GLIMMIX; SAS Institute, 2012; Table 2) to test for relationships between one or more tree variables and parasitoid capture numbers (i.e., total number captured per trap each year) and capture rates. Capture numbers were compared using a negative binomial distribution with the log link function, and capture rates were compared using a binary distribution with the logit function. Degrees of freedom were calculated using the Kenward-Rodger method. Each model included year as a fixed effect, one or more tree variables as covariates, interaction terms, and tree within site as a random effect. We selected tree variable(s) for each model based on published research and *a priori* knowledge of parasitoid biology. Consecutive runs were conducted for each model, and after each run, the year × covariate interaction with the highest non-significant *P*-value ($P \geq 0.05$) was removed until all non-significant interactions were removed. Next, we continued reducing each model by removing the covariate with the highest non-significant *P*-value after each consecutive model run until only significant covariates and/or year × covariate interactions remained in the model (SAS Institute, 2006). When year × covariate terms were significant, models were run for individual years with the significant covariate term as a fixed effect for comparing least squares means.

For each parasitoid species, we used generalized linear mixed models (PROC GLIMMIX, SAS Institute, 2012) to test for relationships between mean parasitoid adults captured in yellow pan traps and mean immature parasitoid brood densities per m^2 of bark surface area during the same season trees were dissected, one season after trees were dissected, and one season before trees were dissected. For each model, we used a Gaussian distribution with site as a random effect. Degrees of freedom were calculated using the Kenward-Rodger method. *Spathius galinae* data for 2016 was excluded from these analyses because releases of this parasitoid did not begin until 2015 and populations were just starting to build (Table 1).

3. Results

3.1. Seasonal occurrence of parasitoids

During 2016–2019, we captured 161 (124 females, 37 males) *T. planipennisi* with yellow pan traps. Captures fluctuated among sample weeks and tended to be greater at the beginning and end of the sampling season each year (Fig. 1). However, significant seasonal trends could not be discerned due to low capture numbers throughout the season during many years. For all years combined, *T. planipennisi* were captured between 111 and 1570 GDD_{10}. Although tree dissections indicated *T. planipennisi* was present at all sample sites each year, no *T. planipennisi* were captured in yellow pan traps at the Harris Nature Center site during two of the sample years.

Yellow pan traps yielded 59 (46 females, 13 males) *S. galinae* during 2016–2019, which were captured between 270 and 1271 GDD_{10} across years. We were unable to discern any seasonal variation or patterns in *S. galinae* emergence or abundance across sample years or sites. No *S. galinae* were captured in yellow pan traps at the Gratiot-Saginaw site in 2017 and 2018 although parasitized larvae were recovered from dissected trees during the same years. In contrast, yellow pan traps

Table 1
Release and establishment history of *Spathius agrili*, *Spathius galinae*, and *Tetrastichus planipennisi* at Michigan study sites.

		Tetrastichus planipennisi		*Spathius agrili*		*Spathius galinae*	
Site	Latitude/ Longitude	First released	Established	First released	Established	First released	Established
Gratiot-Saginaw	43.2337–84.4477	2009	Yes	2007	No	2015	Yes
Harris Nature Center	42.6965−84.3752	2008	Yes	2008	No	2015	Yes
Legg Park	42.6940−84.3822	No release	Yes	No release	No	No release	Yes

Table 2
Effects of tree variables on capture numbers and capture rates of *Spathius galinae* and *Tetrastichus planipennisi* in yellow pan traps. Different tree variables were included as covariates with sample year as a fixed effect in each of 11 GLMMs. Significant covariates and interactions that were statistically significant after all non-significant covariates and interactions were systematically removed are listed for each model (see *Data analysis* in *Methods* section).

	Significant variables after model reduction			
	Tetrastichus planipennisi		*Spathius galinae*	
Model number and covariates	Capture numbers[a]	Capture rates[b]	Capture numbers	Capture rates
1. Fresh woodpecker feeding holes	NS[c]	NS	NS	NS
2. DBH	DBH × year (P=0.0268)	NS	NS	NS
3. Canopy dieback	NS	NS	No convergence	NS
4. Crown class	Crown class (P=0.0508)	Crown class (P=0.0078)	NS	NS
5. Fresh woodpecker feeding holes + Canopy dieback	NS	NS	NS	NS
6. Fresh woodpecker feeding holes + Sprouts	NS	Sprouts × year(P=0.0361)	NS	NS
7. Fresh woodpecker feeding holes + Sprouts + Crown class	Crown class (P=0.0508).	Crown class (P=0.0078)	NS	NS
8. Crown class + DBH	DBH × year (P=0.0166); Crown class(P=0.0156)	Crown class (P=0.0078)	NS	NS
9. Fresh woodpecker feeding holes + Crown class + DBH	DBH × year (P=0.0166); Crown class(P=0.0156).	Crown class (P=0.0078)	NS	NS
10. DBH + Bark texture	DBH × year (P=0.0268).	NS	NS	NS
11. DBH + Bark texture + Crown class	DBH × year (P=0.0166); Crown class(P=0.0156)	Crown class (P=0.0078)	No convergence	NS

[a] Capture numbers represent mean number of individuals captured in yellow pan traps.
[b] Capture rates represent mean number of positive traps. NS[c] = No variables significant.

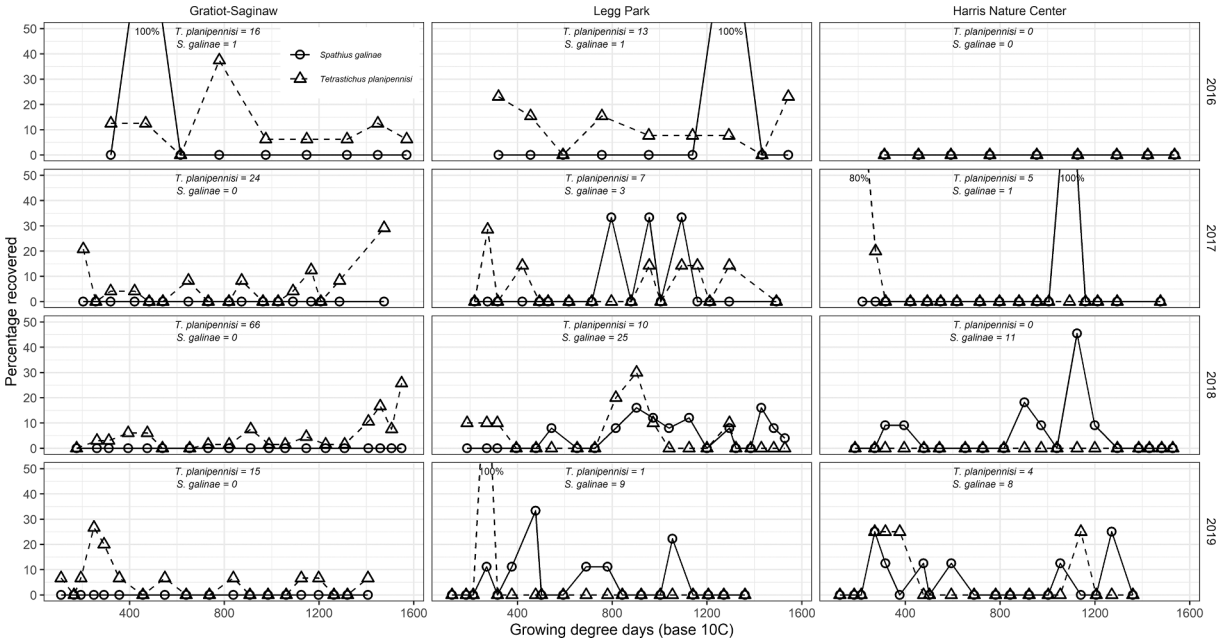

Fig. 1. Percentage of *Tetrastichus planipennisi* (indicated by triangle markers) and *Spathius galinae* (indicated by circle markers) captured in yellow pan traps by growing degree day (base 10 °C) at three sites over four years.

captured *S. galinae* at the Legg Park site in 2016 and 2017, while tree dissections did not yield *S. galinae* parasitized larvae during the same years.

3.2. Host tree variables related to parasitoid captures

Tetrastichus planipennisi recoveries in 2016 were significantly higher on trees with signs or symptoms of recent EAB attack compared to trees without (DF = 1; X^2 = 8.05; P = 0.0046). For all 11 analyses of covariance models, *T. planipennisi* capture numbers varied significantly among years (results from reduced model that only included year: DF = 3, 85; F = 20.73; $P < 0.0001$; Table 3). *Tetrastichus planipennisi* capture numbers were highest in 2017 and 2018, and lowest in 2016 and 2019 (Table 3). Models that included the covariates tree DBH and tree crown class, showed a significant effect of tree crown class (DF = 1, 70; F = 6.14; $P = 0.0156$) and the interaction of year × tree DBH (DF = 4, 153; F = 3.13, $P = 0.0166$) on *T. planipennisi* mean captures (Table 2). Models that included the covariate tree crown class, but not tree DBH, showed a marginally significant relationship between tree crown class (DF = 1, 84; F = 3.93; $P = 0.0508$) and *T. planipennisi* capture numbers. *Tetrastichus planipennisi* capture numbers were highest on suppressed/intermediate trees, followed by dominant/codominant trees, and lowest on open grown trees (Table 4). Mean captures of *T. planipennisi* significantly increased with decreasing tree DBH in 2018 (DF = 1, 15; F = 4.90; $P = 0.0428$), but no significant relationship was found for 2016 (DF = 1, 118; F = 1.58; $P = 0.2114$), 2017 (DF = 1, 28; F = 0.52; $P = 0.4788$), or 2019 (DF = 1, 28; F = 0.05; $P = 0.8247$) (Fig. 2). All other covariates tested were not found to be significantly related to mean *T. planipennisi* capture numbers (Table 2).

Similar to the results for capture numbers, year had a significant

Table 3
Tetrastichus planipennisi and *Spathius galinae* least squared mean capture numbers and capture rates among different sampling years. Differences in least squared means among years were compared using a GLMM. A negative binomial distribution and log link function were used for capture numbers and binary distribution with the log function for capture rates. Least squared means followed by the same letter are not significantly different ($P < 0.05$).

	Capture numbers[a]	Capture rates[b]
Species/year	Mean ± SE	Mean ± SE
Tetrastichus planipennisi		
2016	0.16 ± 0.11c	0.16 ± 0.11b
2017	0.76 ± 0.55ab	0.22 ± 0.05a
2018	1.31 ± 0.94a	0.22 ± 0.04a
2019	0.43 ± 0.32b	0.20 ± 0.90ab
	DF 3,85; F 20.7; P < 0.0001	DF 3,206; F 5.14; P 0.0019
Spathius galinae		
2017	0.03 ± 0.05b	0.03 ± 0.05a
2018	0.29 ± 0.39a	0.19 ± 0.22a
2019	0.18 ± 0.25a	0.19 ± 0.22a
	DF 2,87; F 5.75; *P* 0.0045	DF 2,87; F 3.05; *P* 0.0527

[a] Capture numbers represent mean number of individuals captured in yellow pan traps.
[b] Capture rates represent mean proportion of positive traps.

Table 4
GLMM comparing tree crown class as a fixed effect on *Tetrastichus planipennisi* least squared mean capture numbers (negative binomial distribution and log function with DBH*year interaction term) and capture rates (binomial distribution and logit function). Least squared means followed by the same letter are not significantly different ($P < 0.05$).

	Capture numbers[a]	Captures rates[b]
Tree crown class	Mean ± SE	Mean ± SE
Suppressed/Intermediate	0.76 ± 0.61a	0.51 ± 0.25a
Codominant/Dominant	0.47 ± 0.37ab	0.33 ± 0.22ab
Edge/Open grown	0.36 ± 0.30b	0.18 ± 0.15b
	DF 2,58; F 3.48; *P* 0.0374	DF 2,88; F 3.63; *P* 0.0305

[a] Capture numbers represent mean number of individuals captured in yellow pan traps.
[b] Capture rates represent mean proportion of positive traps.

effect on *T. planipennisi* capture rates for all models (DF = 3, 206; F = 5.14; $P = 0.0019$; Table 3). Models that included tree crown class showed a significant effect of tree crown class (DF = 1, 97; F = 7.39; $P = 0.0078$) on *T. planipennisi* capture rates. *Tetrastichus planipennisi* capture rates were higher on suppressed/intermediate trees, followed by dominant/codominant, then open grown trees (Table 4). For the model that included sprouts but not crown class, the interaction of year × sprouts was significantly related to *T. planipennisi* capture rates (DF = 4, 202; F = 2.62, $P = 0.0036$). In 2016, *T. planipennisi* capture rates increased significantly with increasing number of sprouts (DF = 1, 98; F = 8.25; $P = 0.005$; Fig. 3). *Tetrastichus planipennisi* capture rates also trended higher with increasing sprout numbers in 2017 and 2018; however, the trend was opposite in 2019.

No tree variables for any of our models that converged were significant for *S. galinae* capture numbers or rates (Table 3). *Spathius galinae* trap capture numbers varied significantly among years (DF = 2, 87; F = 5.75, $P = 0.0045$) with 2018 and 2019 having the highest captures and 2017 having the lowest capture numbers. In addition, year was marginally significant for *S. galinae* capture rates (DF = 2, 87; F = 3.05; $P = 0.0527$).

3.3. Tree dissections

Tetrastichus planipennisi was recovered from dissected trees or

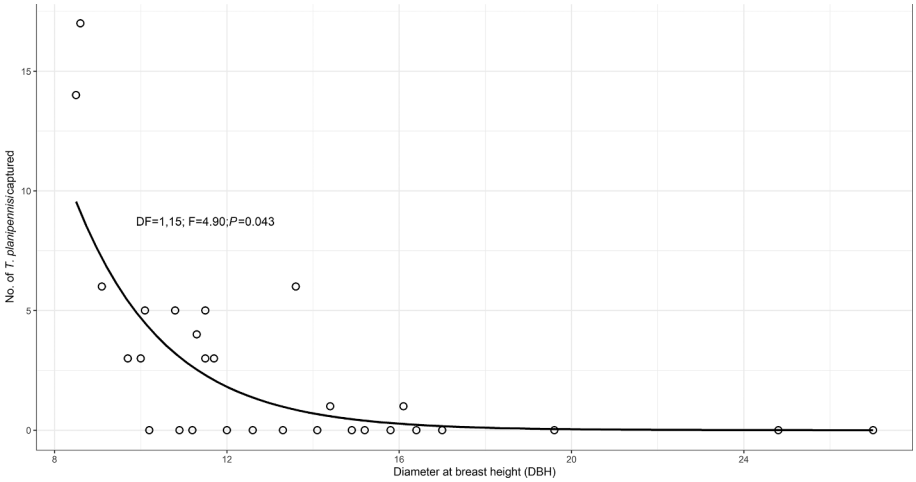

Fig. 2. Number of *Tetrastichus planipennisi* captured in 2018 in yellow pan traps as a function of tree diameter at breast height. Regression line was fit using a negative binomial distribution.

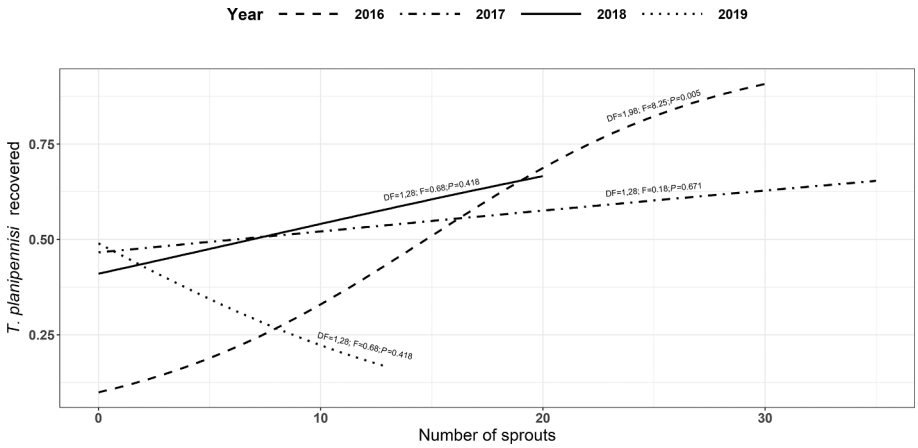

Fig. 3. Regression lines fit to proportion of yellow pan traps with positive *Tetrastichus planipennisi* captures (data points not shown) as a function of number of epicormic sprouts on sampled trees by year. Regression lines were fit to a binary distribution.

saplings at all sites in 2015–2018. *Spathius galinae* was recovered from dissected trees at the Gratiot-Saginaw site in 2016, from Gratiot-Saginaw and Harris Nature Center in 2017, and all sites in 2018. The number of *T. planipennisi* parasitized EAB larvae/m^2 of ash phloem dissected from trees or saplings was significantly related to adult *T. planipennisi* trap captures the following year (DF = 1, 9; F = 6.38; P = 0.0324; Fig. 4). However, the number of immature *T. planipennisi* parasitized EAB larvae/m^2 of ash phloem dissected was not significantly related to adult *T. planipennisi* trap captures the same year (DF = 1, 6; F = 0.03; P = 0.8609), or adult captures the year before (DF = 1, 3; F = 0.23; P = 0.6596). The number of EAB larvae parasitized by *S. galinae* per m^2 of phloem was not significantly related to the number of *S. galinae* adults captured in traps the year following (DF = 1, 6; F = 0.05; P = 0.8664), year of, (DF = 1, 3; F = 1.91; P = 0.2566), or year after (DF = 1, 3; F = 3.30; P = 0.3204) tree dissections (Fig. 4).

4. Discussion

Yellow pan traps are frequently used to monitor the establishment of introduced EAB parasitoids, but their capture efficiency for monitoring introduced larval parasitoids is often poor. Understanding parasitoid seasonal abundance and the relationship of parasitoid captures to host tree variables and parasitized larval densities could improve yellow pan trap efficacy as a monitoring tool. During our study, yellow pan traps captured both EAB larval parasitoids throughout the growing season, although capture numbers and capture rates were low for *T. planipennisi* and these metrics were even lower for *S. galinae*. Our results indicate that certain tree variables and parasitized EAB larval densities from the prior year were related to *T. planipennisi* adult captures, but no variables were related to *S. galinae* captures.

Although *T. planipennisi* were captured in yellow pan traps during our study, capture numbers and rates were quite variable across sites and years. In fact, yellow pan traps did not recover *T. planipennisi* at Harris Nature Center in 2016 and 2018, even though *T. planipennisi* parasitized larvae were confirmed at these sites during these same years. In January 2019, a severe cold wave in the upper Midwest was associated with high levels of freeze-mortality of immature *T. planipennisi* and may explain the lower captures of *T. planipennisi* during 2019 (Duan et al., 2020). Lower capture numbers and rates in 2016 were not associated with a severe weather event but may have been influenced by the different sampling protocol used in the first year of the study. Although we used 40 traps at each study site in 2016, compared to only 10 in subsequent years, we had not prioritized trap placement on ash trees that displayed EAB signs or symptoms as was done in 2017–2019. Of the 161 *T. planipennisi* that were collected during 2016–2019, only 29 were collected in 2016 (Fig. 1).

At our study sites, *T. planipennisi* captures were inconsistent throughout the growing season, but there was a slight tendency for captures to be higher near the initiation and the termination of the trapping period. It is likely that adults were active before deployment and after removal of traps during some years, given that Abell et al. (2016) recovered *T. planipennisi* using sentinel larval logs as early as 226 GDD_{10} and as late as 1717 GDD_{10}. When we averaged GDD_{10} for the earliest female *T. planipennisi* captures each sample year and subtracted the total yearly GDD_{10} accumulations, *T. planipennisi* should have averaged at least 2.5 generations per year at our trapping locations [development rates from Duan et al. (2018b) and temperature data from weather monitoring stations within 10 km of study sites downloaded from Michigan State University Enviroweather (2019)]. Despite this, we did not see substantial increases in trap captures as trapping seasons progressed, or any consistent generation peaks across sites and years. *Tetrastichus planipennisi* does not undergo diapause and may enter winter at any immature stage depending on when cold temperatures arrest development of the last seasonal generation. Depending on the severity of winter temperatures, a range of immature stages may successfully overwinter and continue development when temperatures warm in the spring, resulting in asynchronous emergence of adults (Duan et al., 2011). Asynchronous emergence combined with a female's reproductive lifespan of more than six weeks (Duan et al., 2011), may partially explain the staggered and fluctuating nature of trap catches and lack of seasonal abundance trends or patterns.

Low capture numbers and rates of *S. galinae* were likely attributed, in part, to the more recent release history of this parasitoid at our study sites (Table 1). Although *S. galinae* were first released at or near our study sites in 2015, these individuals were several weeks old and had been exposed to EAB larvae multiple times prior to field releases. Nevertheless, one *S. galinae* was captured in a yellow pan trap at Gratiot-Saginaw in 2016 prior to initiation of releases in 2016, suggesting that *S. galinae* successfully established at this site after its first release in 2015. *Spathius galinae* releases continued in 2016–2017 using only naïve adults and higher *S. galinae* capture numbers and rates in 2018 and 2019, relative to 2017, are likely a result of population increases following *S. galinae* releases and establishment. As with *T. planipennisi*, high freeze-induced mortality of immature *S. galinae* was documented in spring 2019 (Duan et al., 2020). However, *S. galinae* yellow pan trap capture numbers and rates did not decrease significantly in 2019 as expected. Absolute values for *S. galinae* capture numbers and rates were lower in 2019 compared to 2018, and we suspect that the overall low *S. galinae* captures limited the ability of our statistical models to detect a significant difference between these two sample years.

Similar to *T. planipennisi, S. galinae* recovery events occurred across a range of GDD_{10}. When we averaged GDD_{10} for the earliest female

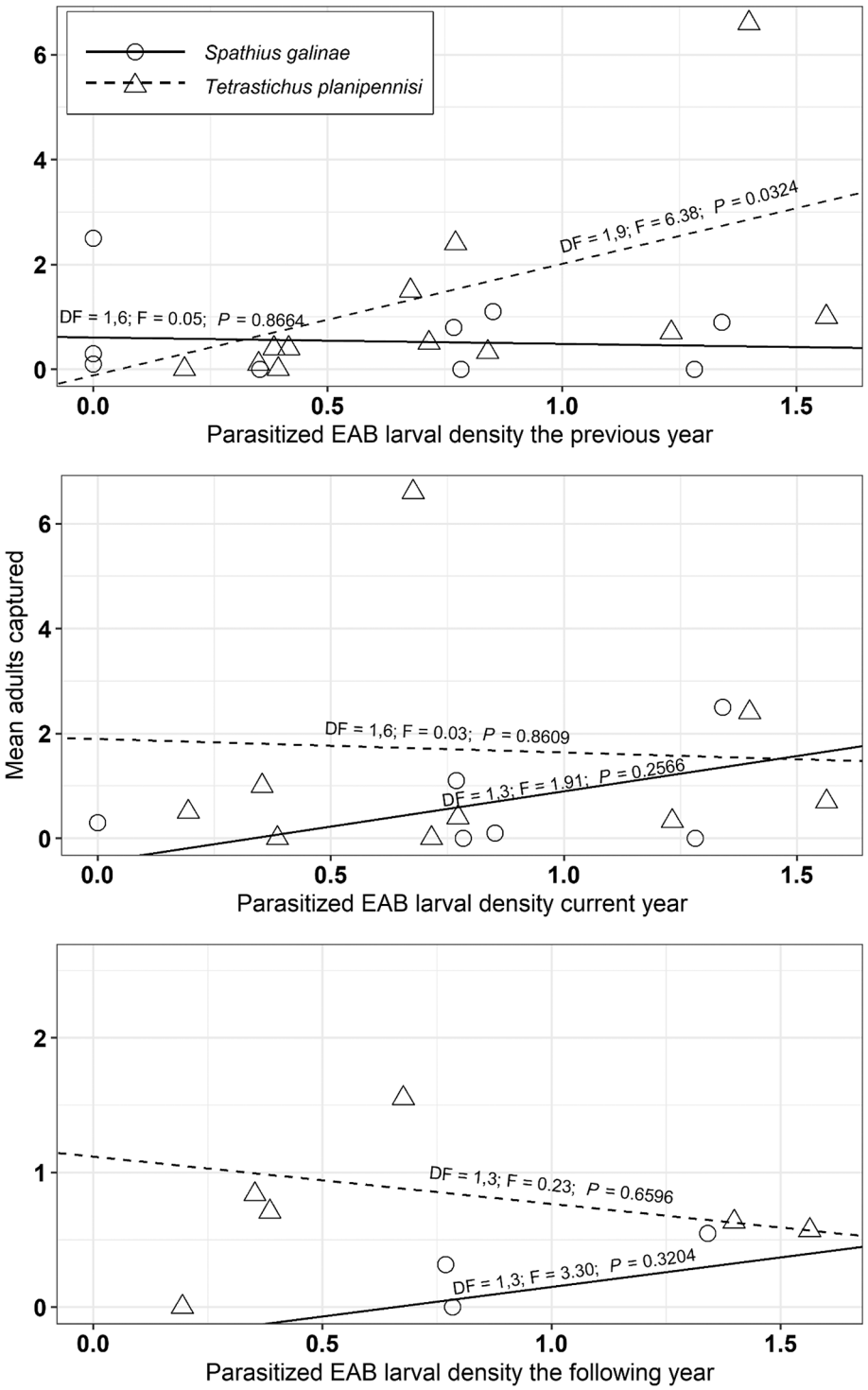

Fig. 4. Relationship between mean number of adult parasitoids captured in yellow pan traps and density of parasitized EAB larvae per m^2 of ash phloem dissected for *Tetrastichus planipennisi* and *Spathius galinae* at three sites over four years.

S. galinae captures for 2018 and 2019 and subtracted the total yearly GDD_{10} accumulations [development rates from Watt et al. (2016) and temperature data from Michigan State University Enviroweather (2019)], *S. galinae* should have averaged 2.1 generations per year at our trapping locations. However, *S. galinae* captures did not substantially increase as the trapping season progressed for any years, nor were there any consistent generation peaks across sites and years. It is unclear if *S. galinae* diapauses, given that both live *S. galinae* larvae in cocoons and mature naked larvae have been dissected from trees at these study sites in early spring (unpublished data). Like *T. planipennisi*, lack of *S. galinae* seasonal abundance trends in yellow pan trap captures may partially be explained by asynchronous emergence of adults if multiple immature stages successfully overwinter.

Some host tree variables were associated with yellow pan trap captures of *T. planipennisi,* but not *S. galinae*. Crown class was the most consistent tree variable related to *T. planipennisi* captures, with higher captures on intermediate and suppressed trees. In general, trees with intermediate and suppressed crowns are smaller in DBH than dominant

and codominant or open grown trees at the same sites, and *T. planipennisi* are primarily limited to EAB larvae in smaller trees due to their short ovipositor (Abell et al., 2012; Duan et al., 2021). Tree DBH did have a significant negative relationship with *T. planipennisi* capture numbers in 2018, which was attributed to two smaller trees capturing large numbers of *T. planipennisi* (Fig. 2). DBH alone was not a significant covariate in any model; however, models that included the interaction of DBH and year, resulted in significant crown class effects on *T. planipennisi* capture numbers (Table 2). Tree DBH in our study ranged from 5.0 to 30.4 cm, but most trees were > 9 cm DBH (Fig. 2). If our study included more sapling-size trees, the effect of tree DBH on *T. planipennisi* trap captures may have been more consistent. Alternatively, other variables associated with intermediate or suppressed trees may have influenced *T. planipennisi* trap captures. For instance, *T. planipennisi* may fly at a lower height under the canopy of intermediate and suppressed trees compared to taller trees, increasing their likelihood of landing in traps that were only 1.75 m above the ground. It is also possible that light conditions and visually contrasting surrounding vegetation may affect visual attractiveness of yellow pan traps attached to intermediate or suppressed trees under closed canopies compared to traps on open tree boles of larger trees (Abrahamczyk et al. 2010).

Given that *T. planipennisi* recoveries were higher on trees with signs and symptoms of recent EAB attack in 2016, we anticipated that both parasitoid species would be more attracted to trees with abundant signs and symptoms of recent EAB attack. However, this was not confirmed by our results. For *T. planipennisi*, the interaction between sprouts and year had a significant effect on capture rates for models that included epicormic sprouts and fresh woodpecker feeding holes. When analyzed separately by year, however, only 2016 *T. planipennisi* capture rates had a significant positive relationship with increasing numbers of epicormic sprouts. Increasing levels of other EAB signs and symptoms, such as fresh woodpecker feeding holes and canopy dieback, were not significantly related to capture rates or numbers for either parasitoid species, although both are strongly correlated with the presence of EAB larvae in trees (Cappaert et al., 2005; Lindell et al., 2008; Flower et al., 2013; Jennings et al., 2016). Thus, in our study, *T. planipennisi* and *S. galinae* yellow pan trap captures did not appear to be correlated with tree infestation levels.

Our finding that *T. planipennisi* yellow pan trap capture numbers were related to parasitized EAB larval densities from the previous year, suggest that captures may potentially provide estimates of *T. planipennisi* parasitism without destructively sampling host trees. The lack of significant relationship between the density of larvae parasitized by *S. galinae* and adult trap captures may be partially a result of the more recent establishment of this parasitoid at our study sites, which also reduced the number of years that adult captures and tree dissections were compared. As *S. galinae* becomes more abundant at release sites, it may become easier to detect relationships between adult trap captures and density of parasitized larvae and other tree and site factors.

It is likely that utilization of yellow pan traps as an EAB parasitoid recovery tool will continue, given their low technology requirements, ability to capture the four introduced parasitoid species, and avoidance of destructive host tree sampling methods. Yellow pan traps captured *T. planipennisi* and *S. galinae* at most sites and in most years of our study when they were documented parasitizing EAB larvae by tree dissections. However, parasitoid capture numbers and rates were often low, especially for *S. galinae,* and this may have limited the ability of our models to detect possible relationships with certain tree variables. Our results suggest that the use of yellow pan traps for determining EAB larval parasitoid establishment would be optimized by sampling throughout the growing season across multiple years on ash trees with signs or symptoms of recent EAB attack, and, for *T. planipennisi*, attaching traps to intermediate or suppressed ash trees of smaller DBH and with abundant epicormic sprouts. Deploying 15 traps per site, as recommended in the Emerald Ash Borer Biological Control Release and Recovery Guidelines (USDA APHIS/ARS/FS, 2021), should also improve recovery success, compared to only 10 traps per site deployed during most years of our study. Negative yellow pan trap recoveries at parasitoid release locations should be interpreted cautiously and, ideally, supplemented with host tree dissections or sentinel larval logs to verify whether parasitoids are present.

CRediT authorship contribution statement

Toby R. Petrice: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Therese M. Poland:** Conceptualization, Resources, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Leah S. Bauer:** Conceptualization, Investigation, Methodology, Resources, Funding acquisition, Writing – original draft, Writing – review & editing. **John S. Strazanac:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Jian J. Duan:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Jonathan M. Schmude:** Data curation, Formal analysis, Investigation. **F. William Ravlin:** Conceptualization, Funding acquisition, Resources, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abell, K.J., Duan, J.J., Bauer, L., Lelito, J.P., Van Driesche, R.G., 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). Biol. Control 63, 320–325. https://doi.org/10.1016/j.biocontrol.2012.08.009.

Abell, K.J., Bauer, L.S., Miller, D.L., Duan, J.J., Driesche, R.G.V., 2016. Monitoring the establishment and flight phenology of parasitoids of emerald ash borer (Coleoptera: Buprestidae) in Michigan by using sentinel eggs and larvae. Florida Entomol. 99, 667–672. https://doi.org/10.1653/024.099.0413.

Abrahamczyk, S., Steudel, B., Kessler, M., 2010. Sampling Hymenoptera along a precipitation gradient in tropical forests: The effectiveness of different coloured pan traps. Entomol. Exp. Appl. 137, 262–268. https://doi.org/10.1111/j.1570-7458.2010.01063.x.

Bauer, L.S., Liu, H., Miller, D., Gould, J., 2008. Developing a classical biological control program for *Agrilus planipennis* (Coleoptera: Buprestidae), an invasive ash pest in North America. Newsl. Michigan Entomol. Soc. 53, 38–39.

Bauer, L.S., Duan, J.J., Gould, J.R., Van Driesche, R., 2015. Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Can. Entomol. 147, 300–317. https://doi.org/10.4039/tce.2015.18.

Belokobylskij, S.A., Yurchenko, G.I., Strazanac, J.S., Zaldívar-Riverón, A., Mastro, V., 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Ann. Entomol. Soc. Am. 105, 165–178. https://doi.org/10.1603/an11140.

Cappaert, D., Mccullough, D.G., Poland, T.M., Siegert, N.W., 2005. Emerald ash borer in North America: a research and regulatory challenge. Am. Entomol. 51, 152–165.

Duan, J.J., Oppel, C.B., Ulyshen, M.D., Bauer, L.S., Lelito, J., 2011. Biology and life history of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a larval endoparasitoid of the emerald ash borer (Coleoptera: Buprestidae). Florida Entomol. 94, 933–940. https://doi.org/10.1653/024.094.0430.

Duan, J.J., Bauer, L.S., Abell, K.J., Lelito, J.P., Driesche, R.V., 2013. Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan:

Potential for success in classical biocontrol of the invasive emerald ash borer (Coleoptera: Buprestidae). J. Econ. Entomol. 106, 1145–1154. https://doi.org/10.1603/EC13047.

Duan, J.J., Bauer, L.S., van Driesche, R.G., Gould, J.R., 2018a. Progress and challenges of protecting North American ash trees from the emerald ash borer using biological control. Forests 9, 1–17. https://doi.org/10.3390/f9030142.

Duan, J.J., Schmude, J.M., Wang, X.-Y., Watt, T.J., Bauer, L.S., 2018b. Host utilization, reproductive biology, and development of the larval parasitoid *Tetrastichus planipennisi* influenced by temperature: Implications for biological control of the emerald ash borer in North America. Biol. Control 125, 50–56. https://doi.org/10.1016/j.biocontrol.2018.06.009.

Duan, J.J., Van Driesche, R.G., Crandall, R.S., Schmude, J.M., Rutledge, C.E., Slager, B. H., Gould, J.R., Elkinton, J.S., Bernal, J., 2019. Establishment and early impact of *Spathius galinae* (Hymenoptera: Braconidae) on emerald ash borer (Coleoptera: Buprestidae) in the Northeastern United States. J. Econ. Entomol. 112, 2121–2130. https://doi.org/10.1093/jee/toz159.

Duan, J.J., Bauer, L.S., Van Driesche, R., Schmude, J.M., Petrice, T., Chandler, J.L., Elkinton, J., 2020. Effects of extreme low winter temperatures on the overwintering survival of the introduced larval parasitoids *Spathius galinae* and *Tetrastichus planipennisi*: implications for biological control of emerald ash borer in North America. J. Econ. Entomol. 113, 1145–1151. https://doi.org/10.1093/jee/toaa048.

Duan, J.J., Van Driesche, R.G., Schmude, J.M., Quinn, N.F., Petrice, T.R., Rutledge, C.E., Poland, T.M., Bauer, L.S., Elkinton, J.S., 2021. Niche partitioning and coexistence of parasitoids of the same feeding guild introduced for biological control of an invasive forest pest. Biol. Control 160, 104698. https://doi.org/10.1016/j.biocontrol.2021.104698.

Duan, J.J., Gould, J., Slager, B.H., Quinn, N.F., Petrice, T.R., Poland, T.M., Bauer, L.S., Rutledge, C.E., Elkinton, J.S., Van Driesche, R., 2022. Progress toward successful biological control of the invasive emerald ash borer in the United States., in: Van Driesche, R.G., R. L. Winston, R.L., Perring, T.M., Lopez, V.M. (Eds.), Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity. FHAAST-2019-05. USDA Forest Service, Morgantown, WV, pp. 232–250.

Flower, C.E., Knight, K.S., Rebbeck, J., Gonzalez-meler, M.A., 2013. The relationship between the emerald ash borer (Agrilus planipennis) and ash (Fraxinus spp.) tree decline : using visual canopy condition assessments and leaf isotope measurements to assess pest damage 303, 143–147.

Haack, R., Jendek, E., Liu, H., Marchant, K.R., Petrice, T.R., Poland, T.M., Ye, H., 2002. The emerald ash borer: a new exotic pest in North America. Newsl. Michigan Entomol. Soc. 47, 1–5.

Herms, D.A., McCullough, D.G., 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. Annu. Rev. Entomol. 59, 13–30. https://doi.org/10.1146/annurev-ento-011613-162051.

Herms, D.A., 2004. Using degree-days and plant phenology to predict pest activity. In: V. Krischik and J. Davidson, eds. IPM (Integrated Pest Management) of Midwest Landscapes, pp. 49- 59. Minnesota Agricultural Experiment Station Publication 58-07645, 316 pp.

Jennings, D.E., Duan, J.J., Bauer, L.S., Schmude, J.M., Wetherington, M.T., Shrewsbury, P.M., 2016. Temporal dynamics of woodpecker predation on emerald ash borer (*Agrilus planipennis*) in the northeastern U.S.A. Agric. For. Entomol. 18, 174–181. https://doi.org/10.1111/afe.12142.

Johnson, T.D., Lelito, J.P., Raffa, K.F., 2014. Responses of two parasitoids, the exotic *Spathius agrili* Yang and the native *Spathius floridanus* Ashmead, to volatile cues associated with the emerald ash borer. *Agrilus planipennis* Fairmaire. Biol. Control 79, 110–117. https://doi.org/10.1016/j.biocontrol.2014.05.004.

Jones, M.I., Gould, J.R., Warden, M.L., Fierke, M.K., 2019. Dispersal of emerald ash borer (Coleoptera: Buprestidae) parasitoids along an ash corridor in western New York. Biol. Control 128, 94–101. https://doi.org/10.1016/j.biocontrol.2018.09.004.

Koenig, W.D., Liebhold, A.M., 2017. A decade of emerald ash borer effects on regional woodpecker and nuthatch populations. Biol. Invasions. 19, 2029–2037. https://doi.org/10.1007/s10530-017-1411-7.

Lindell, C.A., McCullough, D.G., Cappaert, D., Apostolou, N.M., Roth, M.B., 2008. Factors influencing woodpecker predation on emerald ash borer. Am. Midl. Nat. 159, 434–444. https://doi.org/10.1674/0003-0031(2008)159[434:FIWPOE]2.0.CO;2.

MapBioControl. 2022. Agent release tracking and data management for federal, state, and researchers releasing four biocontrol agents released against emerald ash borer. [WWW Document]. URL http://www.mapbiocontrol.org/ (accessed 12.19.2022).

McCullough, D.G., Siegert, N.W., 2007. Estimating potential emerald ash borer populations (Coleoptera: Buprestidae) using ash inventory data. J. Econ. Entomol. 100, 1577–1586.

Michigan State University Enviroweather, 2019. Weather-based pest, natural resources, and production tools. Michigan State University, East Lansing, Michigan [WWW Document]. URL https::://www.enviroweather.msu.edu/ (accessed 1.1.20).

Parisio, M.S., Gould, J.R., Vandenberg, J.D., Bauer, L.S., Fierke, M.K., 2017. Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biol. Control 106, 45–53. https://doi.org/10.1016/j.biocontrol.2016.12.009.

Petrice, T.R., Bauer, L.S., Miller, D.L., Stanovick, J.S., Poland, T.M., Ravlin, F.W., 2021. Monitoring field establishment of the emerald ash borer biocontrol agent *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae): sampling methods, sample size, and phenology. Biol. Control 156, 104535. https://doi.org/10.1016/j.biocontrol.2021.104535.

Poland, T.M., McCullough, D.G., 2005. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. J. For. 1990, 118–124.

Rutledge, C.E., Van Driesche, R.G., Duan, J.J., 2021. Comparative efficacy of three techniques for monitoring the establishment and spread of larval parasitoids recently introduced for biological control of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). Biol. Control 161, 104704. https://doi.org/10.1016/j.biocontrol.2021.104704.

SAS Institute, 2006. Analysis of repeated measures data. In: SAS for Mixed Models. Cary, NC, pp. 160–202.

SAS Institute, 2012. OCUser's Manual, Version 9.4. SAS Institute, Cary, NC.

USDA APHIS/ARS/FS, 2021. Emerald ash borer biological control release and recovery guidelines. USDA APHIS/ARS/FS, Riverdale, Maryland.

Vinson, S.B., 1998. The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biol. Control 11, 79–96. https://doi.org/10.1006/bcon.1997.0601.

Ward, S.F., Liebhold, A.M., Morin, R.S., Fei, S., 2021. Population dynamics of ash across the eastern USA following invasion by emerald ash borer. For. Ecol. Manage. 479, 118574.

Watt, T.J., Duan, J.J., Tallamy, D.W., Hough-Goldstein, J., Ilvento, T.W., Yue, X., Ren, H., 2016. Reproductive and developmental biology of the emerald ash borer parasitoid *Spathius galinae* (Hymenoptera: Braconidae) as affected by temperature. Biol. Control 96, 1–7. https://doi.org/10.1016/j.biocontrol.2016.01.011.

Yang, Z.-Q., Yao, Y.-X., Wang, X.-Y., Strazanac, J.S., 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* haliday (Hymenoptera: Eulophidae). Proc. Entomol. Soc. Washingt. 108, 550–558.