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Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer

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HIGHLIGHTS

- Yellow pan traps (YPTs) can recover introduced EAB biological control parasitoids.
- YPTs can be as effective as destructive tree sampling for *O. agrili* and *S. agrili* detection.
- YPTs can be as effective as sentinel log traps for *S. agrili* detection.
- The minute egg parasitoid *Oobius agrili* can be repeatedly recovered from YPTs.
- YPTs can recover *O. agrili* and *T. planipennisi* 20 m from the point of release.

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ABSTRACT

Emerald ash borer (*Agrilus planipennis* Fairmaire, EAB) is an invasive forest pest and the target of an extensive biological control program designed to mitigate EAB-caused ash (*Fraxinus* spp.) mortality. Since 2007, hymenopteran parasitoids of EAB from northeastern Asia have been released as biological control agents in North America, including *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid; *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a larval endoparasitoid; and *Spathius agrili* (Hymenoptera: Braconidae), a larval ectoparasitoid. Following parasitoid releases in new locations, methods currently used to document presence and establishment and to monitor dispersal of parasitoids in the field were simultaneously evaluated, including destructive sampling of entire trees and deployment of egg sentinel logs (ESLs), egg sentinel cups (ESCs), larval sentinel logs (LSLs), and yellow pan traps (YPTs). All three parasitoids were recovered using YPTs and destructive sampling of trees. *Spathius agrili* was the only species to be recovered using LSLs, however, results indicate YPTs were as effective as LSLs. YPTs were also as effective as destructive sampling of entire trees for *O. agrili* and *S. agrili* detection. YPT trap catches were significantly associated with egg parasitism on sampled trees by *O. agrili*, but not for larval parasitoids. Additional research indicated YPTs are effective in recovering *O. agrili* and *T. planipennisi* at distances as great as 20 m from release points. It is therefore recommended that YPTs be used as the preferred method for parasitoid recovery as the other methods are much more labor intensive and prone to difficulties.

1. Introduction

The emerald ash borer (EAB, *Agrilus planipennis* Fairmaire, Coleoptera: Buprestidae) is an invasive wood-boring beetle responsible for the deaths of hundreds of millions of ash trees (*Fraxinus* spp.) in North America (Cappaert et al., 2005; Herms and McCullough, 2014, emeraldashborer.info 2016). Given the current scale of infestation, management of EAB in forest settings now primarily relies on a biological control program intended to provide long-term population suppression and mitigate future ecological impacts of EAB to the greatest extent possible (Bauer et al., 2015).

Exploratory surveys for natural enemies within areas of EAB's native range in northeastern China and the Russian Far East have resulted in the identification of four major hymenopteran parasitoids: *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae),

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an egg parasitoid (Zhang et al., 2005); *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), a larval endoparasitoid (Liu et al., 2003; Yang et al., 2006); and the larval ectoparasitoids *Spathius agrili* Yang (Yang et al., 2005), and *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) (Belokobylskij et al., 2012). Following research on EAB population dynamics in China and host specificity testing (Federal Register, 2007, 2015), these species were approved for release in the United States and are now mass reared for release by USDA (Federal Register, 2007, 2015; Bauer et al., 2015). Releases of *O. agrili*, *T. planipennisi*, and *S. agrili* began in 2007, and *S. galinae* releases in 2015.

Since 2007, both *T. planipennisi* and *O. agrili* have been recovered in nine states and *S. agrili* in six states (Mapbiocontrol, 2016), demonstrating the ability of these introduced parasitoids to reproduce in the field and survive at least one winter over a large geographic area. Establishment of biological control agents, defined as sustained populations for at least two years after final field release in an area, has been confirmed for *O. agrili and T. planipennisi* at long-term study sites in Michigan and other states (Abell et al., 2014; Bauer et al., 2015; Duan et al., 2013, 2015). Despite the apparent ability to overwinter in some northern states, *S. agrili* has not been documented to persist more than two years, possibly due to asynchrony with its host. Long-term monitoring of parasitoids after release is essential to determining successful establishment and evaluating impacts of parasitoids on EAB populations as well as ash survival and regeneration. During initial establishment of parasitoids, small populations make field recovery difficult and so great effort has been invested in developing effective parasitoid detection and monitoring methods.

One sampling method to detect presence of EAB parasitoids involves felling EAB-infested ash trees, cutting them into logs, incubating logs in cardboard rearing tubes, and identifying emerging adult parasitoids (Bauer et al., 2011; Abell et al., 2015; Gould et al., 2016). This method isn't conducive for documenting egg parasitism as *O. agrili* is very small, and so an additional step is needed to examine the bark of infested ash trees for EAB eggs, followed by debarking and collecting/dissecting EAB larvae (Abell et al., 2014; Duan et al., 2013, 2015). As the EAB biological control program expands in North America, more efficient non-destructive monitoring methods are needed for detection of all EAB biocontrol agents in the field to evaluate their establishment and spread.

The use of sentinel eggs or larvae, reared from EAB in the laboratory and placed in the field to attract egg or larval parasitoids, is another method of monitoring parasitoid establishment (Bauer et al., 2011; Duan et al., 2012; Jennings et al., 2014). However, rearing and preparing EAB eggs and larvae for use as sentinels is time-consuming, technically challenging, and restricted to those with the knowledge, facilities, and materials required to successfully rear EAB.

Colored pan traps are widely used collecting method for insects and have been used previously to survey parasitic hymenopterans (Noyes, 1989; Pucci, 2008). High-reflectance colors like yellow or white perform well and yellow pan traps (YPTs) have been used successfully to recover EAB parasitoids (Abell et al., 2015; Bauer et al., 2011, 2016; Vrdoljak and Samways, 2012). This method is relatively inexpensive, requiring purchase and assembly of plastic yellow bowls with zip ties and a shelf bracket, and does not require a laboratory or explicit technical skills.

While the aforementioned methods have all been used to recover parasitoids, they were used in independent experiments and to our knowledge no one has carried out side by side field comparisons. Thus, the primary objective of this study was to evaluate these methods simultaneously to determine which are most effective at capturing EAB parasitoids. In addition to comparing these methods, we also used data from destructively sampling trees to determine if any of the methods could be used to indicate parasitism within the trees to which traps were attached.

Although recovery of adult larval parasitoids in YPTs has been documented (Bauer et al., 2011, 2016), we also wished to confirm a more recent observation (J Gould) that the egg parasitoid, *O. agrili*, can also be reliably recovered in these traps. Finally, the effective range of YPTs was investigated to determine whether these traps can recover parasitoids at greater distances not immediately adjacent from the original release point.

2. Materials and methods

2.1. Comparison of parasitoid recovery methods

2.1.1. Release sites

Two parasitoid release sites ~2 km apart were selected near Selkirk in Albany County, NY within mid-successional, EAB-infested forest stands ~5 ha in size and dominated by white ash (*Fraxinus americana* L.). A stand on Currey Ave (N 42.552229, W −73. 846312) consisted of ~75% white ash and showed signs of moderate EAB infestation. A stand on West Yard Rd (N 42.566105, W −73.863208) also consisted of ~75% white ash, however trees already exhibited signs of a more advanced infestation, including visible symptoms of EAB-induced dieback and some tree mortality.

A single cluster of 10 live ash trees with clear signs of EAB infestation, especially woodpecker foraging, was selected at each of the two sites. Trees were girdled in mid May 2013 by removing a 15 cm strip of bark from the trunk at a height of 1 m to attract EAB emerging in Jun and Jul, thereby concentrating within-tree EAB populations and increasing the number of EAB eggs and larvae available for parasitoids (McCullough et al., 2009). Girdled trees had a mean DBH of 9.4 cm (±0.6 SE, range 6.8–13.0 cm) at Currey Ave and 11.7 cm (±0.5 SE, range 7.8–13.9 cm) at West Yard Rd.

2.1.2. Parasitoid releases

Oobius agrili were disseminated from release devices placed at a height of 1.5 m on the 10 girdled ash trees at both field sites on two occasions (20 total release devices) in Jun/Jul of 2013. *Oobius agrili* were received from the USDA-APHIS-PPQ Biological Control Rearing Facility in Brighton, MI as late-instar larvae or pupae inside parasitized EAB eggs laid on filter paper. Filter paper was placed in a field release device modeled after "Oobinators" (See USDA APHIS/ARS/FS 2016), with minor modifications to structure but not overall function. Approximately 2400 parasitized EAB eggs were deployed in total. At each site, 600 eggs were deployed at the beginning of the experiment, coinciding with the beginning of the EAB egg laying season (~2–3 weeks after adult EAB emergence), and an additional 600 eggs were deployed two weeks later (Currey Ave on 21-Jun and 6-Jul, West Yard Rd on 28-Jun and 12-Jul). Parasitized eggs were distributed evenly among 10 release devices so that each contained ~60 parasitized eggs and *O. agrili* completed development within the release devices under natural ambient conditions. To encourage survival, the inside surface of release devices were provisioned with streaks of honey to serve as an initial food and liquid resource for emerging *O. agrili* adults (L Bauer). Six weeks after release, filter paper was collected and eggs examined under a dissecting microscope for *O. agrili* exit holes to quantify the actual number of adult parasitoids to successfully emerge.

Spathius agrili and *T. planipennisi* were both disseminated weekly from 10 emergence logs at each site over a four week period from Aug through early Sept 2013 (note that this time period did not overlap with release of *O. agrili*). Larval parasitoids were received from the Brighton USDA-APHIS-PPQ Biological Control

Rearing Facility as late-instar larvae or pupae inside parasitized EAB larvae in small ash bolts ~15 cm long and ~5.5 cm in diameter. They were attached to non-girdled ash trees dispersed throughout the two release sites. Larval parasitoids completed development and emerged as adults under these natural conditions. A new set of emergence logs was received and placed at both sites weekly for four consecutive weeks. The goal was to release sufficient numbers of both larval parasitoids and preliminary estimates from the Brighton lab indicated ~1200 *S. agrili* and ~3340 *T. planipennisi* were expected to emerge from bolts deployed at each site. This number was recalculated once all parasitoids had emerged by removing the outer bark of emergence logs to count individual *S. agrili* cocoons with exit holes and estimate *T. planipennisi* numbers based on the actual number of EAB larvae parasitized and the mean brood size and number of broods per log. Mean brood size was generated using fully-developed broods of *T. planipennisi* adults that failed to emerge (n = 68) from logs (mean = 32 per parasitized larvae).

2.1.3. Parasitoid recoveries

Egg sentinel logs (ESLs) used for *O. agrili* recovery were prepared using methods adapted from those reported in Duan et al. (2012) using small white ash logs (~15 cm long, ~5.5 cm in diameter) with freshly laid EAB eggs on the bark surface. Adult female EAB for egg production were collected from local ash logs placed in rearing tubes with emerging adults being collected every 1–2 days and transferred to plastic rearing containers (12 × 12 × 15 cm). To facilitate EAB mating, 1–2 males and 3–5 females were kept in each container and dead adults were replaced as needed to maintain these relative numbers. Each ESL was wrapped in 5 mm wide purple curling ribbon to create concealed oviposition sites preferred by EAB females and placed in a container with mated females for 3–5 days prior to placement in the field. Eggs laid beneath the ribbon were counted and circled with fine-point permanent marker prior to being placed in the field. Only 16 ESLs were produced (due to difficulties encountered), with egg densities ranging from 3 to 21 EAB eggs per log (n = 140 eggs total). *Oobius agrili* prefers freshly laid EAB eggs (Duan et al., 2014), therefore we made certain that EAB eggs were <5 days old at the time they were placed in the field. Eggs on ESLs were exposed to parasitism for a one week period and ESLs were replaced weekly for four weeks from late Jun through mid Jul 2013. Due to low ESL availability, all ESLs produced in a given week (3–5) were placed on girdled trees at a single site. Sites were alternated weekly rather than distributing limited ESLs between both sites. As ESLs were collected, eggs were counted and examined under a dissecting microscope to assess parasitism.

Egg sentinel cups (ESCs) were also tested for *O. agrili* recovery (Abell et al., 2015). These were similar to the devices used to release *O. agrili*, however, the inner cups were provisioned with 20 fresh (<5 days old), non-parasitized EAB eggs on small pieces of filter paper overnighted from the Brighton rearing facility and deployed the following day to serve as potential hosts for actively seeking *O. agrili* females. One ESCs were attached to each of the 10 girdled trees at each site on the same date as ESLs were first deployed (28-Jun 2013) and used to monitor for *O. agrili* parasitism weekly for four weeks (n = 80 ESC samples). EAB eggs were replaced with fresh eggs weekly and examined under a dissecting microscope two weeks after being collected from the field (to allow for development of any *O. agrili* larvae) to visually assess them for parasitism.

Larval sentinel logs (LSLs) used in *S. agrili* and *T. planipennisi* recovery were small white ash logs (~15 cm long,~5.5 cm diameter) provisioned with late instar EAB larvae (Abell et al., 2015). We insured all logs used as LSLs had thin bark and a diameter of <11.2 cm to accommodate the short ovipositor of *T. planipennisi* (Abell et al., 2012). LSLs were infested with EAB larvae by evenly distributing an average of six eggs on filter paper around the upper portion of a log and securing them to the bark beneath a strip of Parafilm®. Batches of 20 logs were placed upright in covered plastic bins (50 × 35 × 30 cm) with ends submerged in 1–2 cm of water to prevent desiccation and maintain humidity. LSLs were incubated at ~31–33 °C for 5 weeks to allow larvae to develop to the 3rd and 4th instar. In total, only 55 of 80 LSLs were successfully infested using this method and able to be used in the field. Prior to deployment, cut ends of LSLs were coated with wood sealant (Anchorseal®) to prevent desiccation. Each set of LSLs were exposed to parasitoids in the field for one week. LSLs were dissected and assessed for healthy and parasitized EAB larvae two weeks after removal from the field (to allow parasitoid larvae to mature to a size visible with the naked eye).

Twenty yellow pan traps (YPTs) were used for recovery of all parasitoid species following methods previously developed and described (Bauer et al., 2016; USDA–APHIS/ARS/FS, 2016). YPTs were attached to all 10 girdled ash trees at the two field sites with a shelf bracket and filled with a 20% clear propylene glycol solution to act as a preservative and two drops of clear non-scented dish detergent to disrupt surface tension. Samples were collected by pouring the contents of YPTs through a mesh paint strainer (mesh size = 226 μm) and examined under a dissecting microscope for the presence of adult parasitoids. YPTs were sampled weekly during the same four week period as all other trap types while parasitoids were being actively released for comparison to other methods (n = 80 YPT samples). Additionally, YPT monitoring continued through 19 Oct 2013, after the other non-reusable trap types were exhausted. Some data are presented for this extended period, however, they were not considered in the actual methods comparisons, and are provided for informational purposes only.

During the *O. agrili* recovery portion of this experiment (28-Jun through 19-Jul), all girdled trees had both YPTs and ESCs and a subset also equipped had ESLs. Each was assigned a position around the trunk of each girdled tree at 90° intervals relative to each other and to the release device. Positions of traps were systematically alternated in relation to the release device to remove placement bias relative to the release devise. On trees with only ESCs and YPTs, a similar placement was used, just without the ESL trap type present. All traps were hung at a height of 1.5 m.

During the *S. agrili* and *T. planipennisi* recovery portion of the experiment (23-Aug through 13-Sept), empty *O. agrili* release devices were left in place for reference and a single LSL and YPT was attached to each girdled tree, occupying positions as described above relative to one another on each tree. Again, only 55 LSLs were produced and so a subset of trees had only YPTs in some weeks. All traps were hung at a height of 1.5 m.

Once all parasitoid recovery was completed, all 10 girdled trees from each site were felled and cut into 1 m bolts and transported to cold storage. A 600 cm^2 cylindrical section of bark, centered on the midpoint of each bolt, was examined under a boom-mounted dissecting microscope and assessed for egg-parasitism by *O. agrili*. Egg parasitism was determined based on the presence of round emergence holes through the dorsal surface or sides of melanized EAB eggs or presence of either wasp pupae or non-emerged adults within (Abell et al., 2014). Following assessment of egg parasitism, the logs were debarked using a drawknife to assess EAB larvae for parasitism by *S. agrili*, *T. planipennisi*, or other possible native parasitoids.

2.1.4. Statistical analyses

Regression analysis was used to determine the relationship between mean egg densities on girdled trees and both total number of parasitized eggs and percentage egg parasitism. Mean egg densities on girdled trees were estimated using the total number

of EAB eggs observed divided by the total surface area inspected. Percentage egg parasitism was calculated using 100 × total number parasitized EAB eggs per tree/total number EAB eggs recovered per tree. A Mann-Whitney *U* test was used for comparisons between both ESLs and LSLs with tree peeling due to non-normally distributed egg and larval density data. A Chi-squared test of independence was used to determine if there was association between positive/negative YPTs and presence/absence of parasitoids in corresponding girdled trees and Cramér's phi was calculated to determine the degree of association. YPTs were considered positive based on recovery of one or more adult parasitoids. Presence of *O. agrili* on girdled trees was defined as the presence of one or more parasitized EAB egg(s). Presence of *S. agrili* and *T. planipennisi* in girdled trees was defined as the presence of developing parasitoid larvae on one or more EAB larva(e). Fisher's exact test was used whenever one or more Chi-square expected values were calculated to be less than five for a given combination of positive/negative and presence/absence. All statistical analyses were performed using Minitab 16. A summary table of all treatments is provided (Table 1).

2.2. Effective range of yellow pan traps

2.2.1. Release site

Parasitoids were released at eight points during summer 2014 within the Reed Road Bird Refuge (53 ha) located in Monroe Co. near Chili, NY. Much of the Reed Road Bird Refuge is a forested wetland with hydric soils supporting facultative wetland plant species, including a canopy with a large green ash (*Fraxinus pennsylvanica* M.) and black ash (*Fraxinus nigra* M.) component. The Reed Road Bird Refuge exhibits advanced signs of EAB infestation, including most large dominant and codominant (mostly green ash) trees already killed by EAB and evidence of extensive woodpecker foraging from several years previous.

2.2.2. Parasitoid release points

Eight parasitoid release points were selected in areas of the refuge with adequate numbers of living ash trees allowing the most accurate deployment of yellow pan traps (YPTs) in rings at two distances, 10 m and 20 m. Central trees used for parasitoid release were chosen so there were 8 ash trees ~10 m away and 16 ash trees ~20 m away arranged in an approximate ring pattern. These 10 m and 20 m rings were each replicated 4 times each for a total of 96 YPTs (n = 32 at 10 m and n = 64 at 20 m).

Distances from each release tree to trees intended for YPT deployment in a given ring were measured, and the 8 or 16 ash trees closest to the desired distance of 10 m or 20 m were selected to serve as YPT trees. YPTs were mounted on selected ash trees using a standard shelf bracket (15 × 20 cm). Release trees were also affixed with a single YPT, placed in close proximity to *O. agrili* release devices and *T. planipennisi* emergence bolts to document emergence and estimate the beginning and end of parasitoid emergence periods for each species throughout the experiment. Mean distance between release trees and YPTs for each ring and mean distance between adjacent YPTs in the same ring are provided in Table 2.

YPTs were attached to either black ash (n = 34) or green ash (n = 62). All trees selected as YPT trees were still alive at the beginning of the experiment and with the exception of several small trees (~4.0–6.0 cm DBH), all were expected to harbor suitable EAB larval hosts given the level of the infestation within the stand.

2.2.3. Parasitoid releases and recoveries

Oobius agrili and *T.* were released using protocols described in Section 2.1.2. Release devices contained 90 *O. agrili*-parasitized EAB eggs and were placed on the release tree at the center of each of the four YPT rings on 2-Jul 2014 once 849 growing degree days (GDD, base temperature 10° C) had accumulated. *Tetrastichus planipennisi* were in 20 emergence bolts, four of which were split in half vertically in order to evenly distribute bolts between all four release points. All surfaces not covered in bark, including ends and exposed wood of bolts which were split, were coated in Anchorseal® green wood sealant to prevent desiccation. Two and one half emergence bolts were placed on the release tree at the center of each YPT ring on 20-Aug 2014 once 1667 GDD-B10C had accumulated. An estimated 575 *T. planipennisi* were expected to emerge at each release point. YPTs were sampled once per week for five weeks following initial deployment of emergence bolts on release trees in the field.

Both *O. agrili* release devices and *T. planipennisi* emergence bolts were left in the field for one week after the end of each portion of the experiment to insure that any remaining parasitoids were allowed additional time to emerge. Once removed from the field, parasitized EAB eggs were examined under a dissecting microscope and *O. agrili* exit holes counted to quantify emergence. The outer bark of *T. planipennisi* emergence bolts was removed and the number of parasitized EAB larvae with *T. planipennisi* exit holes in the overlying bark was counted. Because almost all broods had successfully emerged, this number was multiplied by the mean number of *T. planipennisi* usually produced from a single EAB larva in a laboratory setting (mean = 35, Liu and Bauer, 2007). *Spathius agrili* was not released in this experiment due to limited availability.

2.2.4. Fixed-area plots

Tree data were collected from circular 200 m^2 fixed-area plots with the parasitoid release tree within each replicate serving as plot center. Species and DBH for all stems ⩾5.0 cm DBH were recorded. From this, stem frequency, stem density, mean DBH, and basal area for black and green ash was calculated.

2.2.5. Statistical analyses

Emergence data for *O. agrili* and *T. planipennisi* were analyzed using one-way ANOVA to determine differences in mean emergence of each species at the release point in each replicate. A two-proportions test was performed to determine whether differences existed between the proportion of parasitoid recoveries from YPTs on either black ash or green ash trees relative to the number of each tree species with YPTs attached. Poisson regression analysis

Table 1
Number of each trap type used and EAB host egg and/or larvae availability for each trap type evaluated during recovery method comparison experiment. Note that YPTs are not provisioned with any form of EAB egg or larval host.

Recovery Method	Total Number Evaluated	Min Hosts Available	Max Hosts Available	Average Hosts Available	Total Hosts Available
Girdled Trees	20	5 eggs, 12 larvae	129 eggs, 407 larvae	43 eggs, 151 larvae	865 eggs, 3019 larvae
Egg Sentinel Log (ESL)	16	3 eggs	21 eggs	8–9 eggs	140 eggs
Egg Sentinel Cup (ESC)	80	20 eggs	20 eggs	20 eggs	1600 eggs
Larval Sentinel Log (LSL)	55	1 larva	5 larvae	2–3 larvae	123 larvae
Yellow Pan Traps (YPT)	80	–	–	–	–

Table 2
Summary measurements of mean distance and range of distances between release trees and YPTs and between YPTs and adjacent YPTs for all replicates.

Replicate	Mean Distance from Center (m) ± SE	Range (m)	Mean Distance from Adjacent YPTs (m) ± SE	Range (m)
10 m–1	10.4 ± 0.5	8.3–12.7	7.6 ± 1.6	2.9–16.8
10 m–2	10.2 ± 0.7	7.4–12.6	7.4 ± 0.7	5.2–10.0
10 m–3	10.7 ± 0.3	9.7–12.2	8.0 ± 0.9	5.2–13.2
10 m–4	10.3 ± 0.3	9.4–11.9	7.7 ± 0.8	4.0–10.4
20 m–1	19.0 ± 0.5	15.2–21.6	7.4 ± 1.0	2.8–17.0
20 m–2	19.4 ± 0.2	18.0–20.5	7.5 ± 1.2	1.6–19.3
20 m–3	21.7 ± 0.7	16.6–25.6	8.8 ± 1.1	2.0–15.3
20 m–4	20.2 ± 0.3	17.2–22.1	7.8 ± 1.0	2.4–17.7

was performed using count data to determine if there was a significant relationship between parasitoid recovery and ash stem frequency or ash basal area surrounding release points. Analyses were performed using Minitab 17 or R version 3.3.2 (R Core Team, 2016).

3. Results

3.1. Comparison of recovery methods

3.1.1. Parasitoid emergence

Adult *O. agrili* emerged from 1849 of 2490 (74.3%) parasitized EAB eggs in the 20 release devices deployed on the girdled trees at the two field sites. Adult *S. agrili* emerged from 1122 of 3310 (33.9%) cocoons counted during dissection of 397 parasitized EAB larvae in the 80 emergence logs, as evidenced by visible exit holes found in cocoon remnants. An estimated 6848 *T. planipennisi* successfully emerged from 214 of 285 (75.1%) parasitized EAB larvae in the 80 emergence logs based on presence of emergence holes through the bark overlying larval galleries.

3.1.2. Parasitoid recovery

Overall, girdled trap trees had the highest percentage of parasitoid recoveries compared to all other methods (Table 3). YPTs followed with overall percentages of positive traps ranging from 11.3% for *S. agrili*, 17.5% for *O. agrili*, and 22.5% for *T. planipennisi*. ESCs and ESLs were unsuccessful on all counts, as were LSLs for *T. planipennisi*. LSLs were somewhat successful for *S. agrili* at 14.5%, however, this was not significantly different that YPTs at 11.3% (Fisher's exact test, $P = 0.799$). Capture details are provided below for each species.

Methods using fresh EAB eggs were unsuccessful in attracting *O. agrili*, as no parasitism of EAB eggs was observed within ESCs or on ESLs at either site. EAB eggs on ESLs placed in the field had a mean density of 338 ± 51 (SE) eggs/m^2 (n = 16, range 116–811 eggs/m^2). During sampling for naturally occurring EAB eggs on girdled trees (n = 20), 10.4 m^2 (21.1%) of total bark surface area was inspected and EAB eggs were present at a mean density of 88 ± 19 eggs/m^2 (n = 20, range 9–307 eggs/m^2). Although egg density was significantly lower on girdled trees than on ESLs (Mann-Whitney *U* test, $P \leqslant 0.001$), successful egg parasitism was observed on 12 of 20 girdled trees and *O. agrili* successfully parasitized 110 of 865 (12.7%) recovered EAB eggs. There was a significant positive linear relationship between the number of parasitized eggs recovered and EAB egg density on girdled trees ($F_{1,18} = 11.9$, $P = 0.003$, $R^2_{Adj} = 36.4\%$, Fig. 1).

Eight of 20 YPTs successfully captured a combined total of 28 of 1849 (1.5%) adult *O. agrili* during the 4 week recovery period and seven additional adult *O. agrili* during the remainder of the season (2-Aug through 19-Oct). Successful recovery of adult *O. agrili* in YPTs was associated with presence of *O. agrili* egg parasitism on the corresponding girdled tree to which a given YPT was attached ($\chi^2 = 9.826$, $df = 1$, n = 80, $P = 0.002$). The effect size of this association was moderate ($\varphi_c = 0.35$).

Tetrastichus planipennisi were recovered from YPTs and girdled trees, but not from LSLs. There were 123 suitable EAB larvae (4th instar and J-larvae at time of peeling) counted during dissection of LSLs, but none were parasitized. Parasitism was observed in 15 of 20 girdled trees with 119 of 2680 (4.4%) suitable host larvae successfully parasitized by *T. planipennisi*. YPTs successfully recovered 35 of an estimated 6848 (0.51%) adult *T. planipennisi* to emerge during the designated 4 week recovery period immediately following release. An additional 83 adult *T. planipennisi* were recovered during the five weeks following the designated recovery period (once other traps had been removed) to quantify how many would be caught over a longer period. There was no association between YPT recovery and presence or absence of *T. planipennisi* within-tree larval parasitism due to frequent recovery in YPTs on trees with no parasitized EAB larvae ($\chi^2 = 0.096$, $df = 1$, n = 80, $P = 0.76$). A significantly higher proportion of females than male *T. planipennisi* were recovered in YPTs (88 vs 30, $Z = -8.67$, $P \leqslant 0.001$).

Table 3
Overall percentage of positive traps for each trap type by parasitoid species.

Recovery Method	Positive for *O. agrili*	Positive for *T. planipennisi*	Positive for *S. agrili*
Girdled Trees	60%	75%	35%
Egg Sentinel Log (ESL)	0	NA	NA
Egg Sentinel Cup (ESC)	0	NA	NA
Larval Sentinel Log (LSL)	NA	0	14.5%
Yellow Pan Traps (YPT)	17.5%	22.5%	11.3%

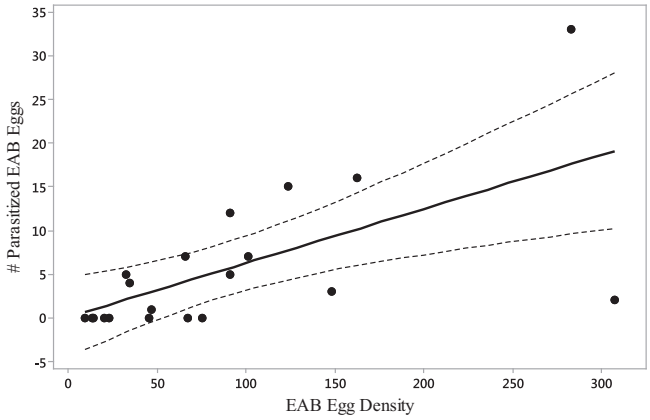

Fig. 1. Responses of *O. agrili* to varying EAB egg densities on girdled ash trees on which they were released ($F_{1,18} = 11.9$, $P = 0.003$, $R^2_{Adj} = 36.4\%$). EAB egg density is given in the number of EAB eggs found on each m^2 of bark. Dashed lines depict 95% confidence intervals.

Spathius agrili parasitized 8 of 123 (6.5%) larvae in LSLs and parasitoid larvae were recovered from 8 of 55 (14.5%) LSLs containing EAB larva(e) suitable for parasitism. LSL larval densities averaged 86.3 ± 6.3 larvae/m^2 and ranged from 38.6 to 231.7 larvae/m^2. *Spathius agrili* larvae were recovered from 7 of 20 (35%) girdled trees and parasitized 10 of 2680 (0.37%) suitable EAB host larvae. Girdled tree larval densities were significantly lower than LSL larval densities, averaging 50.4 ± 9.8 larvae/m^2 and ranging from 4.7 to 159.0 larvae/m^2 (Mann-Whitney *U* test, $P \leqslant 0.001$). No significant difference existed between the overall proportion of positive LSLs and positive girdled trees (Fisher's exact test, $P = 0.179$). Successful recovery of *S. agrili* parasitized EAB larvae in an LSL was not associated with presence or absence of larval parasitism in the corresponding girdled tree to which the LSL was attached (Fisher's exact test, $P = 0.416$).

YPTs recovered 18 (1.7%) of 1122 *S. agrili* that successfully emerged over the entire season. No significant difference existed between overall proportion of positive YPTs and LSLs (Fisher's exact test, $P = 0.799$). Successful recovery of adult *S. agrili* from YPTs was not associated with recovery of EAB larvae parasitized by *S. agrili* in corresponding LSLs (Fisher's exact test, $P = 0.082$). Successful recovery of adult *S. agrili* in YPTs was associated with presence of *S. agrili* larval parasitism in the corresponding girdled tree to which a given YPT was attached (Fisher's exact test, $P = 0.048$). The effect size of this association was low ($\varphi_c = 0.24$).

3.2. Effective range of yellow pan traps

3.2.1. Parasitoid emergence

A total of 3008 (83.6%) *O. agrili* adults emerged from parasitized EAB eggs placed at all release points, with a mean emergence of 376 ± 24 (SE) individuals at each release point. With the exception of one replicate, where two release devices were destroyed by wildlife, there were no significant differences in total emergence among replicates.

An estimated total of 1155 *T. planipennisi* adults emerged from all emergence bolts so that 144 ± 19 (SE) emerged at each release point. Upon dissection of emergence bolts, exit holes created by adult parasitoids confirmed *T. planipennisi* had successfully emerged from 33 parasitized EAB larvae. No significant differences in mean emergence for *T. planipennisi* were observed among replicates.

3.2.2. Parasitoid recovery

Fifteen *O. agrili* were recovered in YPTs, all of which were associated with the four 20 m release plots (Table 4). Eleven *O. agrili* were caught in release tree YPTs and four in ring perimeter YPTs 20 m away. The first recovery of a single *O. agrili* occurred on 16-Jul 2014 from a release tree YPT. Ten *O. agrili* were recovered from two release tree YPTs the following week on 23-Jul 2014, nine of which came from just one of these YPTs. This same week, four *O. agrili* were recovered from ring perimeter YPTs in three of the four 20 m replicates. Two of these individuals were recovered from separate, non-adjacent ring perimeter YPTs in the same 20 m replicate. No additional *O. agrili* were recovered in release tree YPTs or 20 m ring perimeter YPTs after 23-Jul 2014. There were no *O. agrili* recovered from release tree or ring perimeter YPTs in any of the 10 m replicates during the entire experiment.

A total of 33 *T. planipennisi* were recovered in YPTs, 22 of which were caught in YPTs on release trees, 4 in YPTs on trees 10 m from release points, and 7 in YPTs 20 m away (Table 4). The first recovery of *T. planipennisi* individuals occurred on 3-Sept 2014, when one was recovered from a release tree YPT and one was recovered from each of two ring perimeter YPTs in separate 20 m replicates. YPTs then recovered *T. planipennisi* for an additional three consecutive weeks. During the week of 10-Sept 2014, 12 *T. planipennisi* were recovered from release tree YPTs in two 10 m replicates and all four 20 m replicates, a single individual was recovered from one 10 m replicate, and three individuals were recovered from three separate traps in two separate 20 m replicates. In the replicate that recovered two individuals, YPTs were adjacent to one another. During the week of 17-Sept 2014, nine *T. planipennisi* were recovered from release tree YPTs in two separate 20 m replicates, two individuals were recovered from ring perimeter YPTs in two separate 10 m replicates, and two individuals were recovered from ring perimeter YPTs in two separate 20 m replicates. During the week of 23-Sept 2014, one *T. planipennisi* was recovered from a ring perimeter YPT in a 10 m replicate.

Oobius agrili was recovered from one ring perimeter YPT on green ash and three ring perimeter YPTs on black ash; however, there was no significant difference between the proportions of positive YPTs to total number of YPTs on each ash species (Fisher's exact test: $P = 0.126$). Similarly, *T. planipennisi* was recovered from three YPTs placed on black ash and eight YPTs placed on green ash and again no significant difference between proportions was observed (Fisher's exact test: $P = 0.366$)

Regression analysis showed *O. agrili* recovery was not correlated with ash stem density ($F_{1,6} = 0.3$, $P = 0.872$), however, there was a significant quadratic relationship between *T. planipennisi* recovery and ash stem density (F1,6 = 7.92, P = 0.037, $R^2_{Adj} = 69.7\%$). Poisson regression indicated a non-significant effect of YPT distance on total parasitoid recovery ($\beta = 0.20$; 95% CI: −1.11, 1.31; $P = 0.77$), but yielded a significant relationship between total parasitoid recovery (*O. agrili* and *T. planipennisi* combined) and total ash basal area within each replicate ($\beta = 2.39$; 95% CI: 1.18, 4.87; $P = 0.016$). This indicates total parasitoid capture increased by 2.39 times for every square meter increase in ash basal area (Fig. 2).

Finally, general direction from release trees to all YPTs where parasitoids were recovered was recorded, demonstrating the greatest numbers of parasitoids were recovered to the north and northeast of release points. A wind rose diagram was adapted using count data to create a visual representation of general patterns of parasitoid dispersal direction (Fig. 3).

4. Discussion

4.1. Comparison of recovery methods

Although the presence of *O. agrili* was confirmed by recovery of parasitized EAB eggs on girdled trees and adult wasps were recov-

Table 4
Parasitoid recoveries from YPTs on release trees and ring YPTs at each distance on by sampling date.

YPT location	*O. agrili* Recovery					*T. planipennisi* Recovery				
	7/9	7/16	7/23	7/30	8/6	8/27	9/3	9/10	9/17	9/23
10 m release tree							1	2	6	
10 m ring								1	2	1
20 m release tree		1	10					10	3	
20 m ring			4				2	3	2	

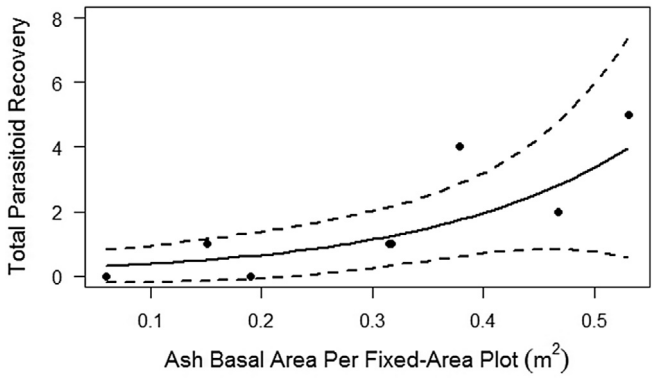

Fig. 2. Rings of yellow pan traps (YPTs) surrounding fixed-area plots with higher ash basal area recovered more parasitoids overall than those with lower ash basal area ($\beta = 2.39$; 95% CI: 1.18, 4.87; $P = 0.016$).

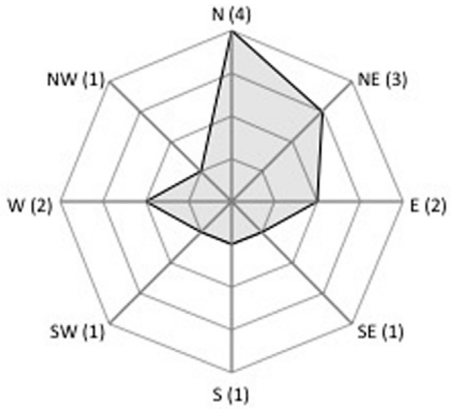

Fig. 3. Combined recoveries of *O. agrili* and *T. planipennisi* and the general direction of dispersal from the release tree to YPTs with parasitoid recoveries. The number in parentheses accompanying each direction label indicates the total number of individual parasitoids recovered in that direction.

ered in YPTs at both release sites in 2013, ESCs and ESLs were unsuccessful in documenting egg parasitism. Unfortunately, ESL sample size was much smaller than desired due to difficulties encountered in making ESLs, i.e. low survivorship of adult EAB in captivity resulted in few sexually mature females for egg production and oviposition. Despite higher mean EAB egg densities on ESLs than girdled trees, even the maximum egg density achieved on an ESL used in this study (811 eggs/m^2) was lower than mean densities (~1265 eggs/m^2) on ESLs used by Duan et al. (2012) during 2009 and 2010. This may mean there were insufficient egg densities necessary to attract or entice *O. agrili* to remain in close proximity long enough for parasitism to occur. It is also possible EAB eggs presented in ESCs and on ESLs were not preferable for *O. agrili* parasitism at the time of exposure, as *O. agrili* has been shown to prefer younger eggs (Duan et al., 2014). To accommodate this, all EAB eggs used were <5 days old when placed in the field and therefore <12 days old at time of collection. However, ambient temperatures >24 °C during Jun 2013 and potentially higher temperatures within ESCs exposed to direct sunlight may have reduced egg preferability to an even shorter time period.

Additionally, *O. agrili* used by Duan et al. (2012) were released as pre-nourished adults. Laboratory reared *O. agrili* have been shown to die within 24 h of emergence unless provided with honey (L Bauer, unpublished data). Though there was emergence of large numbers of adult *O. agrili*, it is possible they failed to locate honey provided within release cups and dispersed away from ESLs in search of alternative food resources on the main trunk or other locations before parasitism could occur.

Though relatively little is known about the flight capabilities of *O. agrili*, association between recoveries of adults in YPTs and presence of parasitized eggs on girdled trees suggest a close relationship between *O. agrili* and its tree of origin. There was only one instance wherein *O. agrili* was recovered in a YPT on a girdled tree from which no parasitized EAB eggs were recovered during sampling. YPTs were less reliable predictors of *S. agrili* or *T. planipennisi* parasitism within girdled trees, as each species was recovered on multiple occasions in YPTs on girdled trees containing no parasitoid larvae. This suggests the larger *S. agrili* and *T. planipennisi* likely disperse away from their natal tree more often, highlighting the potential role that proximity to abundant host-bearing ash trees may play in efficacy of YPTs for different parasitoid species.

Both LSLs and YPTs proved to be successful methods of *S. agrili* recovery and were no less effective at detecting presence of *S. agrili* than destructive sampling of entire girdled trees. Because only one EAB larvae was parasitized in each positive LSL, further correlation could not be established between parasitism rates in LSLs and girdled trees. Although YPTs did not provide information on parasitism rates in this study, our results indicate YPTs are as effective as LSLs for simple detection of *S. agrili*. Given these results, it is also promising that YPTs might be effective for monitoring *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae), another recently-described and approved biological control agent of EAB now being released in a limited number of states (Federal Register, 2015).

Unlike *S. agrili*, *T. planipennisi* was not recovered using LSLs and was only recovered in YPTs. Although it was not likely that *T. planipennisi* females were physically prevented from ovipositing through thin bark of small LSLs, i.e. bark did not exceed their ovipositor length (Abell et al., 2012), no *T. planipennisi*-parasitized larvae were found. Previous studies using similar bolts have demonstrated successful parasitism of LSLs by *T. planipennisi* in MI (L Bauer et al., 2011); however, LSLs used to recover *T. planipennisi* during those trials contained late-instar larvae manually inserted into small cavities excavated beneath bark flaps, which is different in that ours contained larvae hatched from eggs placed on logs. Bauer et al. (2011) also indicated LSLs with manually inserted larvae were preferred by *T. planipennisi* to those with naturally developing larvae, indicating either a change in host apparency or that females were able to access larvae more easily through the bark flaps when that method was used.

When hosts are limited, female *S. agrili* have also been shown to exclude *T. planipennisi* females in lab and field trials by means of more efficient host location and unwillingness of *T. planipennisi* to utilize larvae immobilized by *S. agrili* parasitism (Ulyshen et al., 2011). This is presumably due to reliance by both species on host vibrations as a way to locate hosts, in which case larvae already paralyzed by *S. agrili* during oviposition would be undetectable to searching *T. planipennisi* females (Ulyshen et al., 2011). Regardless, in this study, *S. agrili* did not parasitize larvae in LSLs to a degree that *T. planipennisi* would have been excluded.

YPTs were the only method besides whole tree dissection to recover all three species of released parasitoids. Unlike female *O. agrili*, which can engage in host feeding on fluids from exposed EAB eggs during oviposition (L Bauer, personal observation), parasitoids of concealed larvae such as *S. agrili* and *T. planipennisi* are presumed to seek floral nectar or insect honeydew for nourishment. Colored pan traps, intended to mimic flower or foliage reflectance, have often been used to collect insects and yellow is often selected for its ability to attract parasitic hymenopterans (Noyes, 1989; Pucci, 2008). In addition to yellow, preferences for white and green landing surfaces have been demonstrated for *S. agrili* (Cooperband et al., 2013). Although color preferences for *O. agrili*

or *T. planipennisi* have not yet been investigated, YPTs recovered similar overall percentages of all three species over the entire season.

At least one *T. planipennisi* was recovered in YPTs each week from 23-Aug 2013 until sampling concluded on 19-Oct 2013. Under laboratory settings, it takes *T. planipennisi* roughly 4 weeks to complete a generation and adult males and females have a median lifespan of 5 and 6 weeks, respectively (Duan et al., 2011). *Tetrastichus planipennisi* females were recovered a full six weeks after the final set of emergence logs were placed in the field. It is possible these were long-lived individuals; however, given the reduced life expectancy of adult insects in the field, we believe YPTs were able to recover some progeny of parasitoids released early in our experiment.

Although a greater number of *T. planipennisi* females than males were caught using YPTs, the ratio of females to males caught is consistent with the expected 3:1 female to male sex ratio observed in laboratory populations (Duan et al., 2011) and so does not indicate differential attraction to YPTs between sexes.

4.2. Effective range of yellow pan traps

Recoveries of *O. agrili* adults in YPTs attached to release trees (n = 8) was not surprising, however, capturing them in traps 20 m from the point of release (n = 64) was unexpected. It is unknown why none were captured in trees ~10 m from dispersal points, however, that they were recovered in traps 20 m away revealed dispersal capabilities greater than previously thought. Though perhaps able to fly directly to ash trees, it is more likely, given the minute size of *O. agrili*, that adults were able to locate ash trees during passive, wind-aided dispersal. Because EAB females oviposit on the bark surface, it is doubtful any induced host tree response occurs until neonate EAB larvae entering the tree begin to feed. Insect eggs typically lack long-range chemical cues (Fatouros et al., 2008) and therefore cues used by egg parasitoids likely originate with the host plant itself, with adult EAB, or a combination of these factors. If *O. agrili* is a passive disperser, cues used in host egg location are likely responsible for informing *O. agrili* when to remain in a potentially host rich environment, rather than attracting *O. agrili* to such an environment from a distance.

Similar to observations made while releasing adults from cups, *T. planipennisi* emerging from emergence bolts appears to have also dispersed rapidly, as evidenced by recoveries of *T. planipennisi* at a distance of 20 m during the first week that individuals also appeared in YPTs on release trees. *Tetrastichus planipennisi* appeared to remain active within release sites throughout the entire sampling period, with recovery of at least one individual at both distances during all four weeks.

With the exception of one release site where green ash was absent in the fixed-area plot, relative abundance of black ash and green ash were similar at all release points. Although black ash is considered highly susceptible to EAB (Rebek et al., 2008), differences in overall health of small black ash and green ash in this study were not apparent. If parasitoid recovery was influenced by attraction to ash trees rather than YPTs, neither *O. agrili* nor *T. planipennisi* appeared to demonstrate preference for ash species and similar numbers of individuals were recovered from YPTs on green ash and black ash for both parasitoid species.

Other characteristics of ash trees surrounding release points in fixed-area plots that might affect recovery and dispersal, such as ash stem density and ash basal area, were predictors of parasitoid recovery in YPTs within the same replicate. Greater frequencies of parasitoid recoveries in replicates with higher ash stem density and ash basal area most likely reflects increased availability of high-quality patches of suitable hosts. Parasitoids frequently encountering high-quality patches of hosts might have reduced incentive to disperse and may have remained within the confines of YPT rings for a longer period of time, thus increasing exposure time to YPTs during weekly sampling periods. Conversely, YPT rings with lower ash stem frequency and ash basal area where parasitoid recovery was low or absent may reflect parasitoids greater tendency to disperse in search of areas of higher ash density, presumably perceived as higher quality host environment by seeking females.

Although cases of dispersal were observed in all cardinal directions, the majority of *O. agrili* and *T. planipennisi* were recovered in YPTs placed on trees to the north or northeast of release points. All YPTs were oriented towards the release point when attached to trees, meaning these YPTs were placed on the southern or southwestern faces of ash trees and would likely have received greater amounts of direct sunlight. This could have resulted in a change in apparency and increased attractiveness of YPTs to dispersing parasitoids. In addition, EAB females are shown to preferentially oviposit on the side of ash trees with greatest exposure to sunlight (Timms et al., 2006). Higher frequency of parasitoid recovery from YPTs oriented in these directions could illustrate a foraging response to increased availability of EAB egg and larval hosts.

5. Conclusions

Although field research involving parasitoids remains difficult to pursue due to inherent challenges associated with observing populations of small and cryptic insects, results of this study indicate YPTs are comparable to several other methods for recovery and require a fraction of the overall effort associated with other more elaborate and sometimes less predictable methods. Compared to other traps, YPTs can be more easily mass-produced, can be produced at a moment's notice, and can be sampled with a high degree of flexibility depending on the user's needs. Most importantly, YPTs are unique in their ability to recover all three species of EAB parasitoids being released during this experiment. Moreover, we found that YPTs can recover *O. agrili* and at greater distances than previously thought.

Recovery of parasitoids from YPTs over several years after release is a good indication that EAB parasitoids are established and attacking EAB. Unfortunately, it was beyond the scope of this study to conclusively document YPT recoveries as indicators of percent parasitism for all parasitoid species and impacts they are exerting on EAB populations. For the purpose of obtaining this information, there is still no better method than dissecting trees. When this is not feasible however, YPTs are recommended as an efficient means of monitoring parasitoid presence and dispersal, especially as states continue to release increasing numbers of parasitoids and new states begin to participate in release programs.

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