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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