Biological Control 60 (2012) 255–261

Contents lists available at SciVerse ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

An improved method for monitoring parasitism and establishment of *Oobius agrili* (Hymenoptera: Encyrtidae), an egg parasitoid introduced for biological control of the emerald ash borer (Coleoptera: Buprestidae) in North America

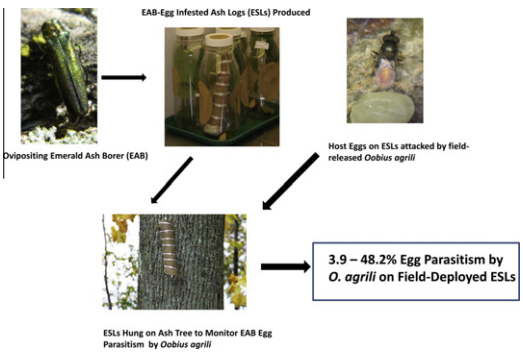
Jian J. Duan [a,*], Leah S. Bauer [b,c], Jason A. Hansen [c], Kristopher J. Abell [d], Roy Van Driesche [d]

[a] USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 19713, United States
[b] USDA Forest Service, Northern Research Station, East Lansing, MI 48823, United States
[c] Department of Entomology, Michigan State University, East Lansing, MI 48824, United States
[d] Department of Plant, Soil, and Insect Sciences, University of Massachusetts, Amherst, MA 01003, United States

HIGHLIGHTS

- *Oobius agrili* is a solitary egg parasitoid of emerald ash borer (EAB).
- It was recently introduced to the USA for biocontrol releases against EAB.
- EAB egg-sentinel ash logs were developed to detect parasitism by *O. agrili*.
- Field deployment of such sentinel logs detected 3.9–48.2% EAB egg parasitism.
- This method is useful for monitoring the establishment and dispersal of *O. agrili*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 April 2011
Accepted 19 November 2011
Available online 14 December 2011

Keywords:
Biological control
Invasive
Exotic
Forest insect pests
Wood borers
Parasitoid sampling
Monitoring
Field detection

ABSTRACT

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a solitary egg parasitoid that has been released in the United States since 2007 for biocontrol of the invasive emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Field and laboratory trials with ash logs infested with EAB eggs were conducted in Michigan between 2009 and 2010 to improve methods for monitoring the establishment of *O. agrili*. Naturally occurring EAB eggs were collected in both parasitoid-release and control (non-parasitoid-release) plots to compare with the EAB egg-sentinel log (ESL) technique. In three parasitoid-release plots, >50% of ESLs had *O. agrili*-parasitized eggs ranging from 3.9% to 48.2% egg parasitism after one week of field exposure. No EAB eggs were attacked by *O. agrili* on the ESLs deployed in control plots. In the laboratory, 100% of ESLs exposed to *O. agrili* inside rearing jars for one week had parasitized-eggs (68.5% egg parasitism). Deployment of ESLs detected low levels of parasitism by *O. agrili* in all three ash stands where *O. agrili* was released in previous years. In contrast, collection of naturally occurring EAB eggs detected the parasitism in only one of these three parasitoid-release ash stands. No parasitism was detected in control ash stands with either method. These findings indicate that populations of *O. agrili* released in previous years had successfully overwintered and established in the released ash stands by 2010, but had not yet dispersed to the control stands.

Published by Elsevier Inc.

* Corresponding author.
E-mail address: jian.duan@ars.usda.gov (J.J. Duan).

1049-9644/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2011.11.007

1. Introduction

The invasive emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has killed tens of millions of ash (*Fraxinus* spp.) trees in both managed and natural forests throughout the midwestern and northeastern United States since its discovery in 2002 in Michigan and Ontario, Canada (Haack et al., 2002; Poland and McCullough, 2006; Michigan State University, 2010; Canadian Food Inspection Agency, 2010). The ash nursery stock industry has collapsed in most of the infested states, and local governments in the affected states have sustained huge phytosanitary costs associated with the ash tree mortality caused by the beetle (USDA APHIS, 2003; Bell, 2005). In addition, ash trees occur extensively in the natural and urban forests of the United States, accounting for nearly 150 million cubic feet of timber products nationwide. It is estimated that there are more than a billion ash trees in the United States (Nowak et al., 2003). Assuming EAB becomes established throughout the range of ash in North America, the cost of EAB to affected communities is estimated to exceed $1 billion per year just for treatment, removal and replacement of infested landscape ash trees (Kovacs et al., 2010).

EAB adults typically begin to emerge in the spring (CAS, 1986; Haack et al., 2002). After emergence, they feed on ash leaves, start mating after ≈ one week and ovipositing ≈ two weeks later (LSB, unpublished data). The eggs are laid in crevices and under bark flakes on limbs and trunks of ash trees. After eclosion, neonate larvae chew through the bark to reach the phloem, where they feed for several months, sometimes developing through two growing seasons before pupating (Cappaert et al., 2005; Wei et al., 2007; Duan et al., 2010a). Adult EAB normally live for 3–6 weeks feeding on mature ash leaves and rarely cause any significant damage to the host tree (Cappaert et al., 2005). Larvae, in contrast, feed for many months on the phloem and outer sapwood, creating extensive galleries that disrupt water and nutrient flow resulting in tree mortality.

Control strategies for EAB shifted from eradication to management, involving the use of integrated approaches to (1) reduce EAB populations in infested areas and (2) slow the spread of EAB to the non-infested areas (Poland et al., 2010; Mercader et al., 2011). These approaches include delimitation of infested areas, regulatory restriction of movement of EAB-infested wood or plant materials, insecticide treatment or physical destruction of infested trees (including artificially girdled EAB trap trees), and biological control via introduction and release of natural enemies collected from EAB's native range (e.g., Liu et al., 2003, 2007; Bauer and Liu, 2007; USDA APHIS, 2007; McCullough et al., 2009; Poland et al., 2010; Mercader et al., 2011). Although none of these approaches alone is sufficiently effective in containing the spread of EAB, biological control via self-propagating and dispersing natural enemies holds promise in reducing EAB populations, particularly in forested ecosystems.

Research on natural enemies of EAB initiated shortly after its discovery in southern Michigan (Liu et al., 2003, 2007; Bauer et al., 2006) and resulted in classical biological control program for EAB using three hymenopteran parasitoids native to northern China (USDA APHIS, 2007). These parasitoids are the EAB egg parasitoid, *Oobius agrili* Zhang and Huang (Encyrtidae) (Zhang et al., 2005) and two species of EAB larval parasitoids, *Spathius agrili* Yang (Braconidae) (Yang et al., 2005) and *Tetrastichus planipennisi* Yang (Eulophidae) (Yang et al., 2006). All three species of exotic parasitoids have now been released in urban and natural ash stands in several EAB-infested midwestern and Atlantic states (USDA APHIS, 2007; Bauer et al., 2008, 2009, 2011; Duan et al., 2010a,b; Gould et al., 2011). Given the extent of on-going field releases, effective methods to evaluate field performance of these newly introduced parasitoids are clearly needed.

As the only egg parasitoid, *O. agrili* is an important component of the EAB biological control program because they reduce EAB egg densities thereby limiting ash phloem damage caused by EAB larval feeding (Liu et al., 2007). In addition, competitive interference by native or introduced parasitoid species is unlikely as egg parasitoids have not been recovered from EAB field populations sampled in Michigan and Pennsylvania (Liu and Bauer, 2007; Duan et al., 2009, 2010a). Largely because of the small size (≈1 mm in adult body size) of this parasitoid and the concealed locations of its host's eggs, which are often laid under loose bark or in crevices of bark, detection of *O. agrili* parasitism and measurement of its impact on EAB populations are challenging and labor intensive. To date, few effective methods have been available to detect parasitism of egg parasitoids of wood-boring buprestid beetles for assessing their dispersal, overwintering, and establishment following biological control introductions.

We recently reported that sampling naturally occurring EAB eggs or deploying laboratory-reared "sentinel EAB eggs" under bark flaps on ash trees could successfully detect low levels of parasitism by *O. agrili* immediately following field release (Duan et al., 2010a). Both sampling methods, however, are labor-intensive, and risk of overlooking or destroying eggs in the process is high. Other methods used to assess overwintering or establishment of *O. agrili* involved placement of field-collected ash logs or bark samples into cardboard-rearing tubes, and trapping emergent *O. agrili* adults in clear collection cups attached to each rearing tube (Bauer et al., 2009, 2010, 2011). Although successful at detecting the presence of *O. agrili*, this method lacks the necessary information on EAB egg densities, and thus cannot quantify the prevalence of EAB egg parasitism. In the present study, our objective was to improve the monitoring methods of *O. agrili* using "egg sentinel logs" (ESL) with freshly laid EAB eggs, which were hung on the trunks of ash trees to determine *O. agrili* prevalence following field release. We also compared the efficacy of this method to that of sampling naturally occurring EAB eggs, as used by Liu et al. (2007) and Duan et al. (2010a), for monitoring EAB egg parasitism in the field.

2. Materials and methods

2.1. EAB adults and production of EAB egg-sentinel logs (ESL)

EAB adults were collected from the trunks of infested green ash (*Fraxinus pennsylvanica* Marsh.) trees in East Lansing, Michigan during June and July 2009 and 2010. In the laboratory, 10 gravid EAB females and several males were placed together in a 3.8-l ventilated plastic jars housed in an environmental growth chamber (16:8 L:D, 65 ± 10% RH, with day and night time temperatures cycling between 25 ± 2 °C and 20 ± 2 °C). Each jar was provisioned with a bouquet of fresh green ash leaves inserted in a vial of water as food for EAB adults, and replaced every two or three days. EAB egg-sentinel logs (ESLs) were made by placing one freshly cut 5-cm diameter × 25-cm long green ash log inside each jar for two or three days. To stimulate EAB egg-laying, curling ribbon (Berwick industries, Berwick, PA) (≈0.5-cm wide) was wrapped snugly in a spiral around each ash log 8–10 times (Bauer and Liu, 2007). Prior to EAB exposure, the spiraled ribbon was outlined with weather-resistant ink to facilitate replacing the ribbon over the EAB eggs after they were counted. Although each rearing jar contained the same number of gravid EAB females, the number of eggs laid on the logs varied considerably, ranging from 10 to 200 eggs. Prior to field deployment, the EAB eggs were counted, and the eggs were rewrapped with the ribbon. Previous observations showed that exposed EAB eggs on infested logs or trees quickly disappeared in the field either from predation or weather-related factors (LSB and JJD, unpublished data). Thus, we recreated this protective egg niche by covering the eggs with the curling ribbon before ESL field deployment.

2.2. Parasitoid

Cultures of *O. agrili*, originating from parasitized EAB eggs collected in Changchun, Jilin Province, China, from 2006 to 2009, are maintained in the USDA FS Northern Research Station Laboratory (East Lansing, MI). This species reproduces by thelytokous parthenogenesis, and although males were also reared from EAB eggs, only females are used for laboratory culture (Liu et al., 2007). Three- to five-day old *O. agrili* adults, originating from parasitized EAB eggs collected in China at different times and reared in the laboratory, were released on EAB-infested ash trees in the release plots.

2.3. Response of O. agrili to EAB egg-sentinel logs

In 2009, the response of *O. agrili* to ESLs was tested in both the field and laboratory. Two study sites, each comprised of a release and non-release control plot located ≈1-km apart, were established in mixed hardwood bottomland forests in Ingham Co., Michigan. One field site contained ≈70% green ash and was located at Legg Park-Harris Nature Center, two contiguous parks in Meridian Township (42°41′N 84°22′W). Prior to this study, 200 adult *O. agrili* were released in the release plot for this site during the 2008 field season. The other field site was located at Crego Park in the City of Lansing (42°43′N 84°32′W) and contained ≈80% green ash. Prior to this study, 118 adult *O. agrili* were released at this site in 2008.

A total of 20 ESLs were deployed, ten at each of the two study sites and divided evenly between the release and control plots (i.e. five ESLs per plot). Each ESL was attached to the trunk of a selected green ash tree in each study plot ≈1.5 m above the ground with an aluminum nail hammered through an eyelet screw affixed in the top of the log. The green ash trees selected for ESL deployment were lightly infested with EAB, lacking overt symptoms of EAB infestation (e.g., bark splits, exit holes, epicormic growth, and woodpecker holes) in the main trunk up to the height of 2 m from the ground. The diameter breast height (DBH) of ash trees used for these studies ranged from 7.5 to 25.0 cm among different stands; the study trees (with ESLs) were separated by 5–300 m within each plot. Each ESL was randomly positioned at the four cardinal directions of EAB-infested ash trees in each release and control plot.

Within 24 h of deploying the ESLs at the two release plots (between June 25 and 27, 2009), we released 60 *O. agrili* on the trunk of each of five selected ash tree, below each ESL and 0.5–1.5 m above the ground. In addition to the field trial, 10 similar ESLs were tested in the laboratory inside 3.8-l jars, as described above. One ESL was exposed to 10 female *O. agrili* in each jar; honey was applied to the screening on the top of each jar as a food source for the parasitoid.

All ESLs were left for seven days on ash trees in the field or test jars in laboratory for exposure to *O. agrili*. Although *O. agrili* parasitizes EAB eggs that are <13 days old in the laboratory, freshly laid EAB eggs are more attractive than older eggs (>7 days) (LSB, unpublished data). Thus, the seven-day exposure period allowed sufficient time for parasitism on ESLs (Duan et al., 2010a). After field deployment or laboratory exposure, each ESL was placed in a ventilated 3.8-l plastic jar, and incubated for approximately eight weeks in a growth chamber (25 ± 2°C; 16:8 L:D, 65 ± 10% RH), for eclosion of *O. agrili* adults. At the end of incubation period, the EAB eggs on each log were recounted and examined under a stereo microscope for evidence of *O. agrili* parasitism. Evidence of *O. agrili* parasitism included the adult exit hole visible on the surface of egg and melanization of eggs, which were dissected to determine if larvae or pupae of *O. agrili* were present. By recounting the eggs from field-deployed ESLs, we determined that <5% of the eggs originally laid on the logs disappeared possibly from predation and/or handling. For data analysis, therefore, we used the final EAB egg count on each ESL to determine percent parasitism.

In 2010, field tests of the response of *O. agrili* to ESLs were replicated at three different Michigan study sites in mixed hardwood bottomland forests from July 10 to August 12. Two field sites were located at Gratiot-Saginaw State Game Area (43°23′N 84°45′W) with ≈50% green ash and East Unit of the Maple River State Game Area (43°08′N 84°32′W) with ≈90% green ash, both in Gratiot County. The third site was at Rose Lake State Wildlife Area (42°48′N 84°20′W) with ≈40% green ash in Shiawassee County. Non-release control plots for this study were not set up in 2010 because establishment of *O. agrili* was not confirmed at these sites, although prior to this study, 374 *O. agrili* adults were released at the Gratiot-Saginaw release site in 2009 (LSB, unpublished data).

At each of the three study sites, six to eight green ash trees with light symptoms of EAB infestation were selected for release of *O. agrili* and deployment of ESLs (one ESL/tree). ESLs were hung on the trunk of each selected ash trees in the similar manner as described above for the 2009 field trial. All ESLs were deployed at the three sites in three days (July 10, 12, and 22); deployment of all ESLs in both parasitoid release and control plots for each site was completed on the same day. Within 24 h of ESL deployment at each site, 120 females of *O. agrili* were released onto the main trunk section of each tree as described above. After two weeks of field exposure, we returned the ESLs to the laboratory for incubation to determine egg parasitism. The additional week of ESL-field exposure provided *O. agrili* the maximum amount of time to find and attack susceptible EAB eggs on the logs. The recount of eggs at the end of the experiments showed ≈3% of the eggs originally laid on the logs had disappeared; the final egg count was used to calculate percent parasitism by *O. agrili*.

2.4. Monitoring establishment of O. agrili

In 2010, the establishment of *O. agrili* in forested EAB biocontrol study sites was evaluated using ESLs. Three study sites, each comprised of a release and non-release control plot located ≈1 km apart, were established in mixed hardwood bottomland forests in Ingham County, Michigan. One study site, located in Central-Nancy Moore Parks (42°43′N 84°25′W) in Meridian Township, contained ≈20% green ash on the edge of a marsh. The second study site, located ≈5 km east in Legg Park-Harris Nature Center (42°41′N 84°22′W) also in Meridian Township, contained ≈70% green ash in a river floodplain. The third study site, located ≈32 km southwest of other study sites was in William M. Burchfield County Park (42°34′N 84°36′W) and contained ≈80% green ash in a bottomland area and 95% white ash in an upland area.

In the Central-Nancy Moore release plot, ≈700 adult *O. agrili* were released in 2007, ≈330 were released in 2008, and ≈300 in 2009. In 2008, we determined that *O. agrili* had successfully overwintered in this plot by sampling two release trees that were felled and cut into sections in March and placed in rearing tubes for emergence of parasitoids, and a total of three *O. agrili* adults were recovered (Bauer et al., 2008). In the release plots for the other two study sites, ≈200 adult *O. agrili* were released in 2008, and ≈300 in 2009. No releases were made in the control plots.

The ESLs were deployed on selected ash trees (one ESL/tree) in the same manner described above for the 2009 field trials between June 24 and July 5, 2010. The ESLs were removed approximately two weeks after field deployment, returned to the laboratory, incubated for at least eight weeks at 25 °C ± 2 °C, 16:8 L:D, 65 ± 10% RH, and scored for parasitism as described above. The recount of eggs at the end of the experiments showed ≈20% of the eggs originally laid on the logs had disappeared; the final egg count was used to calculate percent parasitism by *O. agrili*.

Table 1
Parasitism of EAB eggs by *O. agrili* on egg sentinel logs (ESLs) deployed in EAB-infested field sites located in Meridian Township (Legg Park-Harris Nature Center) and Lansing (Crego Park), Ingham Co., Michigan, in 2009 or ESLs enclosed in rearing jars containing gravid *O. agrili* and in the laboratory.

Test Arena	*O. agrili* release treatment	ESLs tested (*n*)	EAB eggs (mean ± SE) per ESL[a]	% ESL with ⩾ one parasitized EAB eggs[b]	% Egg parasitism[c]
Open field/ash stands	Yes (60 wasps/tree)	10	72.1 ± 22.1a	70a	3.9b
	No (no wasps released)	10	48.9 ± 7.9a	0b	0c
Enclosed lab/rearing jar	Yes (10 wasps/jar)	10	69.9 ± 14.9a	100a	68.5a

[a] Means followed by the same letter are not significantly different from each other (LSD Student's *t* test, type I error rate = 0.05).
[b] Numbers followed by the same letter are not significantly different from each other (Likelihood ratio Chi-square tests, type I error rate = 0.05).
[c] Means followed by the same letter are not significantly different from each other (LSD Student's *t* test, type I error rate = 0.05).

Table 2
Parasitism of EAB eggs by *O. agrili* on egg sentinel logs (ESLs) deployed in EAB-infested field sites located in Maple River and Gratiot Saginaw State Game Areas, both in Gratiot Co., Michigan, and Rose Lake Wildlife Area in Shiawassee County Michigan, in 2010.

Study Sites[a]	ESLs tested (*n*)	EAB eggs (mean ± SE) per ESL[b]	% ESL with ⩾ one parasitized EAB eggs[c]	% Egg parasitism[d]
Gratiot Saginaw	5	11.6 ± 5.4b	80a	48.2a
Maple River	8	51.5 ± 5.1a	75a	14.6b
Rose Lake	8	19.5 ± 2.9b	50a	14.1b

[a] 100 adults of *O. agrili* were released on each tree below where the ESL was hung.
[b] Means followed by the same letter are not significantly different from each other (LSD Student's *t* test, type I error rate = 0.05).
[c] Numbers followed by the same letter are not significantly different from each other (Likelihood ratio Chi-square tests, type I error rate = 0.05).
[d] Means followed by the same letter are not significantly different from each other (LSD Student's *t* test, type I error rate = 0.05).

In addition to deployment of ESLs, we sampled naturally occurring EAB eggs on ten green ash trees (DBH range from 7.5 to 25.0 cm) randomly selected in each parasitoid-release and control plot. The main trunk (0.5–2.0 m above the ground) of each selected ash tree was searched for 30 min by one observer or 15 min by two observers for naturally occurring EAB eggs. The amount of time two observers spent looking for EAB eggs at release and control plots was approximately the same at each study site. Naturally occurring eggs were found by visually searching between bark layers for eggs; eggs were then collected by removing pieces of bark (each ≈0.25 cm^2) to which they were attached (Duan et al., 2010a). EAB eggs on small bark flakes were individually placed into 1.5-ml Eppendorf® snap-cap tubes, returned to the laboratory, and incubated in the tubes at 25 °C ± 3 °C, L:D 16:8 h, and ambient humidity for at least eight weeks for eclosion of *O. agrili* adults. At the end of incubation period, unhatched eggs were dissected under a dissecting microscope for *O. agrili* that were either dead or in diapause (Liu et al., 2007). Percent parasitism by *O. agrili* was calculated based on total number of eggs collected from the sampled trees.

2.5. Statistical analysis

Mean EAB egg densities on ESLs tested in different field and laboratory trials were analyzed using analysis of variance (ANOVA) and partitioned with the least square difference (LSD) Student's *t* test. Likelihood Chi-square was used to test the null hypothesis that the response of *O. agrili* to ESLs (i.e., proportion of sentinel ash logs with presence of one or more *O. agrili*-parasitized EAB eggs) was independent from parasitoid release treatments. Percent EAB egg parasitism by *O. agrili* for each ESL was calculated with 100× (number of parasitized EAB eggs/total number of EAB eggs per log), and then were arcsine-transformed to normalize data distribution for ANOVA. For ESLs tested in both field and laboratory trials in 2009, differences in percent egg parasitism (arcsine-transformed parasitism) by *O. agrili* among field (parasitoid release vs. no release) and laboratory exposure treatments were analyzed with a completely randomized one-way ANOVA model, and means in percent parasitism for different treatments were partitioned by the least square difference (LSD) Student's *t* tests. For the 2009 field trial, additional two-way ANOVA (for split-plot design) was conducted to detect the

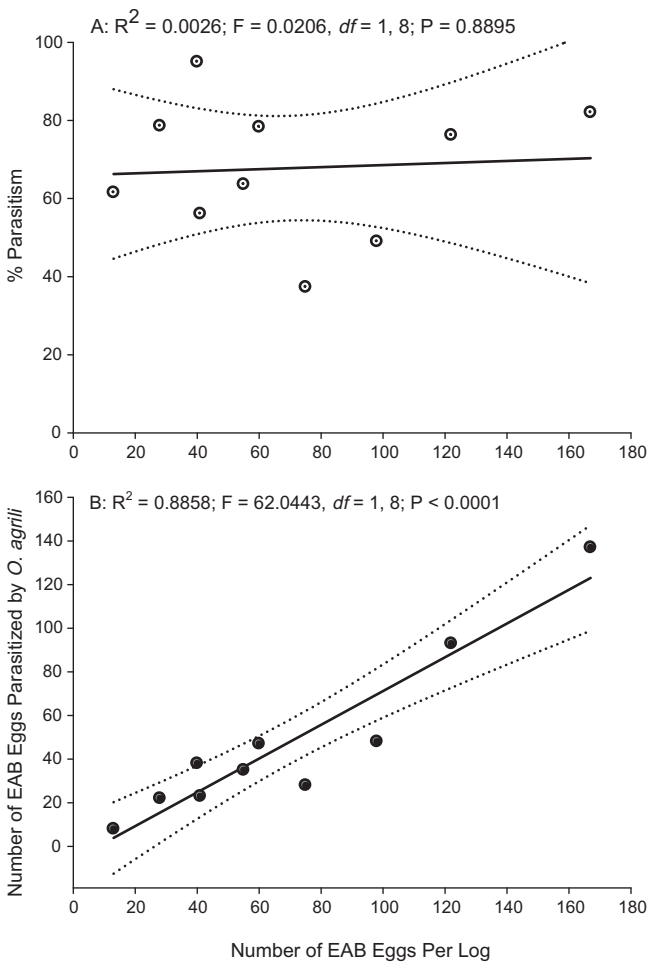


Fig. 1. Responses of *O. agrili* to different densities of EAB eggs on ash logs (ESLs) confined to laboratory rearing jars: (A) host attack rate (% parasitism) in relation to egg density on the log (*n* = 10 logs) and (B) number of parasitized eggs in relation to egg density on the log (*n* = 10 logs). Dotted lines represent 95% confidence interval bands.

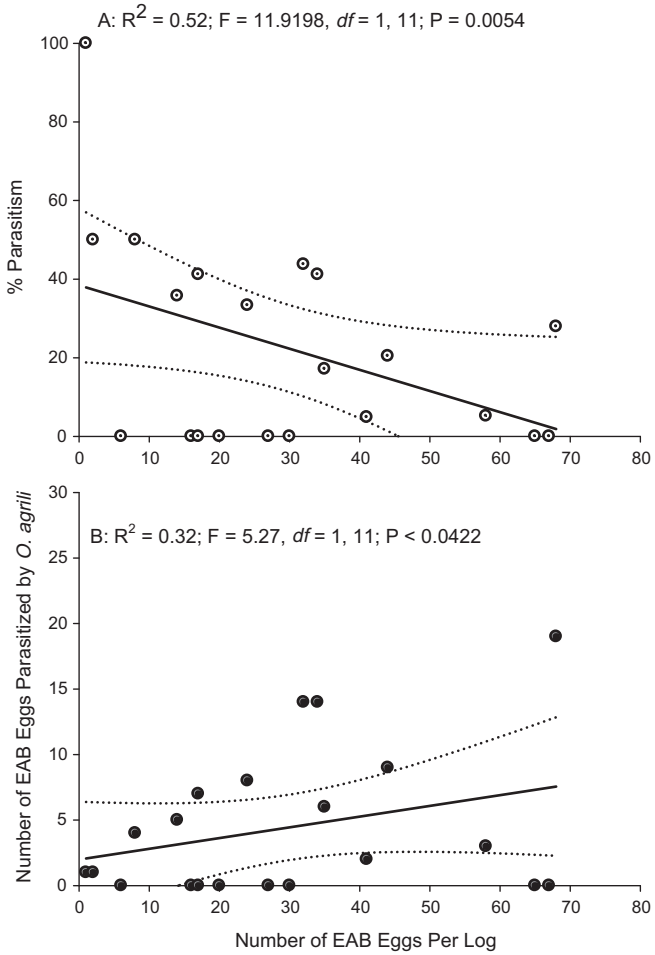


Fig. 2. Responses of field-released *O. agrili* to different densities of EAB eggs on ash logs (ESLs) deployed in natural forests with EAB-infested ash trees (2010, Michigan): (A) host attack rate (% parasitism) in relation to egg density on the log (n = 21 logs) and (B) number of parasitized eggs in relation to egg density on the log (n = 21 logs). Dotted lines represent 95% confidence interval bands.

effect of the study site (main plot) and parasitoid-release treatment (split plot) on mean EAB egg densities and percent parasitism between the two study sites. For field trials in 2010, ANOVA models for the split-plot design were used to detect significant effects of the study sites (main plots – two in 2009 and three in 2010) and treatments (slit-plots: parasitoid release vs. non-release control areas) on percent parasitism. For naturally occurring eggs, percent parasitism was calculated based on the total number of eggs collected from each tree, and then arcsine-transformed for data analysis with the same ANOVA model used for sentinel logs. In the ANOVA models, each ESL or sampled ash tree within the sub-plots was considered a sampling unit. Untransformed means and associated standard errors (SE) were used for presentation of results. All statistical analyses were performed using JMP 8.0.1 (SAS Institute Inc., 2008). In addition to the above analysis, the relationship of *O. agrili* parasitism and EAB egg density on ESLs was analyzed using linear regression model for both 2009 laboratory exposure assay and 2010 field release trial.

3. Results

3.1. Responses of O. agrili to EAB egg-sentinel logs (ESLs)

The mean EAB egg densities on ESLs tested in 2009 (Table 1) ranged from 48.9 to 72.1 eggs per log (bark surface areas = 392.5 cm^2), and there were no significant differences in EAB egg densities on those logs tested among laboratory and field trials (F = 0.6348, df = 2, 27; P = 0.0537). While no EAB eggs were attacked by *O. agrili* on ESLs deployed in the non-release control plots, 70% of the ESLs deployed in the parasitoid-release plots contained one or more EAB eggs parasitized by *O. agrili*, resulting in 3.9% mean egg parasitism. In contrast, 100% of the ESLs exposed to *O. agrili* in laboratory rearing jars contained one or more parasitized EAB egg, resulting in 68.5% mean egg parasitism. Additional analysis of field data (Table 1) showed a marginally significant difference in EAB egg densities on ESLs deployed between the two study sites (Legg Park-Harris Nature Center and Crego Park) (F = 4.29, df = 1, 17; P = 0.0538), but not between the parasitoid release and control treatments (F = 1.15, df = 1, 17, P = 0.2982). A significant difference in percent parasitism by *O. agrili* was detected by ANOVA between parasitoid release and non-release control (F = 9.25, df = 1, 17; P = 0.0074), but not between the two study sites (F = 0.1842, df = 1, 17; P = 0.6732).

In the 2010 field trial, there was a significant difference in EAB egg densities on ESLs deployed at different study sites (F = 22.3010, df = 2, 18; P < 0.0001). ESLs at the Maple River site had significantly higher EAB egg densities than those at the Gratiot Saginaw and Rose Lake sites, which were similar (Table 2). Across all the sites, 50–80% of ESLs had one or more parasitized EAB egg, and egg parasitism among the three study sites ranged from 14.1% to 48.2% (Table 2). Percent EAB egg parasitism by *O. agrili* was significantly higher on sentinel logs deployed in Gratiot Saginaw than the other two areas (F = 4.2312, df = 2, 18; P = 0.0312), between which there was no significant difference.

Results from the linear regression analysis showed that there was no consistent relationship between percent parasitism (host egg attack rate) by *O. agrili* and host egg density on ESLs tested in the laboratory (Fig. 1A) or natural ash stands (Fig. 2A). While there was no significant linear relationship between percent parasitism and host egg density on logs tested in the laboratory assay, there was a significant inverse relationship in the field trial. There was a significant positive linear relationship between the number of EAB eggs parasitized by *O. agrili* and egg density of logs in both laboratory (Fig 1B) and field tests (Fig. 2B).

3.2. Monitoring establishment of O. agrili

In 2010, ESLs deployed in the field had a range of means from 25.3 to 74.8 EAB eggs per log (Table 3). Although no egg parasitism was detected on the ESLs deployed in the non-release control plots, 20–67% of the ESLs deployed in the release plots contained one or more EAB eggs parasitized by *O. agrili*, with a range of 0.4–5.9% egg parasitism (Table 3). When data from all the study sites were analyzed together, differences in percent of ESLs containing parasitized EAB eggs were statistically significant between the release and non-release control plots (Likelihood ratio χ^2 = 5.901, df = 1, P = 0.0151). However, there were still no significant differences in percent parasitism between the parasitoid release and control sites (F = 2.2774, df = 1, 21; P = 0.1449). Nevertheless, these results support that *O. agrili* released in previous years had successfully overwintered and established in the release plots, but had not yet dispersed to the control plots.

The mean number of naturally occurring EAB eggs found per 30-min search by a single observer varied significantly from 5.4 to 17.0 eggs per tree between different study sites (F = 5.9385, df = 2, 44; P = 0.0052), but not between the parasitoid release and control plots (F = 0.3589, df = 1, 42, P = 0.5522) (Table 3). While no parasitized EAB eggs were observed from the control plots, 30% of sampled trees contained one or more *O. agrili*-parasitized eggs in one of the three parasitoid-release plots, which resulted in 2.4% egg parasitism (Table 3). In addition, when data from all study sites were analyzed together, the difference in percent of ash trees containing naturally occurring EAB eggs parasitized by *O. agrili* was statistically signifi-

Table 3
Detection of *O. agrili* parasitism in 2010 of EAB eggs on egg sentinel logs (ESLs) deployed one year after the last field release in Burchfield Park, Legg Park-Harris Nature Center, Central-Nancy Moore Parks in Ingham Co., Michigan, or by collections of naturally occurring or "wild" EAB eggs on infested ash trees at the same sites.

Sampling methods	Study site	Parasitoid releases in previous year	ESLs or ash trees sampled (n)	EAB eggs per ESL or tree (mean ± SE)	% ESL or trees with ⩾ one parasitized eggs	% Egg parasitism
EAB ESLs	Burchfield	Yes	6	25.3 ± 5.9	33.3	0.4
		No	6	26.3 ± 7.1	0	0
	Legg	Yes	3	74.8 ± 13.0	66.7	5.9
		No	3	59.0 ± 32.5	0	0
	Central	Yes	5	43.0 ± 2.5	20.0	4.8
		No	4	39.0 ± 11.5	0	0
	All sites combined	Yes	14	47.2 ± 7.9a	28.6a	3.2a
		No	13	37.3 ± 8.5a	0.0b	0.0a
Survey of wild EAB eggs	Burchfield	Yes	10	17.0 ± 5.5	30	2.4
		No	10	13.9 ± 2.7	0	0
	Central	Yes	10	5.4 ± 0.9	0	0
		No	10	5.75 ± 2.03	0	0
	Legg	Yes	10	4.8 ± 1.1	0	0
		No	10	12.3 ± 3.6	0	0
	All sites combined	Yes	30	10.8 ± 1.7a	10a	1.6a
		No	30	8.7 ± 1.7a	0b	0b

cant between parasitoid release and control plots (Likelihood ratio $\chi^2 = 4.4317$, $df = 1$, $P = 0.0377$); however, there was no significant difference in percent parasitism of naturally occurring EAB eggs by *O. agrili* ($F = 3.0688$, $df = 1, 39$; $P = 0.0873$).

4. Discussion

Few effective methods are available to detect or estimate *O. agrili* parasitism in EAB eggs following its environmental release because the adult parasitoids are minute and develop inside EAB eggs, which are also small and concealed between layers of bark or in bark crevices of ash trees. The findings from this study showed that deployment of egg sentinel logs (ESLs) attached to the trunks of ash trees successfully detected parasitism by *O. agrili* immediately following field release, as well as the year following field release. Our results also indicated that deployment of EAB egg-sentinel logs was more effective than collecting naturally occurring EAB eggs at low *O. agrili* densities in terms of time and labor needed for detection of parasitism.

A recent study by Duan et al. (2010a) investigated the effectiveness of deploying sentinel EAB eggs directly on trunks of selected ash trees to detect parasitism by *O. agrili*, and showed that this method was no more effective at detecting parasitism than collecting naturally occurring eggs. In the past, we either reared egg parasitoids from naturally occurring EAB eggs collected from ash trees (Liu et al., 2007) or from field-collected ash logs or bark samples held in cardboard rearing tubes (Bauer et al., 2009). Although the simplest, the latter method is time consuming and requires destructive sampling of dwindling numbers of ash trees at the biocontrol release sites. We now recommend deployment of ESLs for monitoring the prevalence, establishment, and/or dispersal of *O. agrili* following its environmental release. However, we point out that future research into the standardization of the size of the log, EAB egg densities and positions hung on a tree will further improve the accuracy of the method in determining *O. agrili* parasitism rates, overwintering, establishment, and dispersal rate in the field.

Results of our study showed that the number of EAB eggs parasitized by *O. agrili* on the ESLs was positively related to the egg density on the logs in both laboratory and field trials. However, the rate of parasitism was not consistently influenced by the egg density on test logs. While the rate of parasitism was not significantly influenced by the egg density on test logs in the laboratory assay, it was inversely related to the egg density in the 2010 field trial. The lack of or inverse relationship between parasitism rate and egg density on test logs was probably an artifact due to the fixed number of *O. agrili* in the confined test arena (e.g., in a rearing jar or on a release tree), which would have in turn led to a maximum threshold of host attack rate under the specific testing conditions. For the purpose of detecting parasitism to evaluate parasitoid establishment, however, sentinel logs deployed in the field should have as many EAB eggs as possible. Further studies are needed to determine the minimum number of ESLs and optimal EAB egg density on each log that would allow for detection of parasitism by *O. agrili*. In addition, it is still necessary to optimize the experimental design and to find ways to reduce the large variability observed in the number of deposited eggs on each ESL by EAB adults.

Considering the low rate of parasitism in the sites where *O. agrili* were released in one or two years before the study, it is not surprising that statistical differences were lacking in percent parasitism between parasitoid release and control plots by either the ESL method or by surveying naturally occurring EAB eggs. However, the field-operation time and labor ($n = 1800$ min, 30 min/per tree/per observer) required for surveying naturally occurring EAB eggs on ash trees (total $n = 60$) was more extensive than field-deployment of ESLs, which took several minutes to hang on ash trees. It is true that production of EAB egg-infested logs in the laboratory (before field deployment) would require supplies of sufficient number of EAB adults. In heavily infested areas such as Michigan, however, collecting wild EAB adults from ash trunks was feasible during the course of this study. Future development of methods to mass rearing EAB in the laboratory will greatly facilitate the supply of adult EABs and production of ESLs.

In general, it takes several years for newly introduced biocontrol agents to establish and increase populations large enough to exert significant impacts on the targeted pests following introduction (e.g., Van Driesche et al., 1998). This is confirmed by the results of our study in which we found EAB egg parasitism by *O. agrili* is low and dispersal limited. The *O. agrili* parasitism rate detected by both EAB egg-infested logs (3.2%) or surveying naturally occurring EAB eggs (1.6%) in this study was considerably lower than parasitism (>50%) reported from China (Liu et al. 2007). This is most likely due to the fact that relatively few *O. agrili* were released at these field sites and there was less than two years at most sites for released parasitoid populations to increase. In time, however, the impact of *O. agrili* is likely to increase as more releases are made and the species' population density increases.

Acknowledgments

We thank Deborah Miller (USDA Forest Service), Tim Watt and Ian Lane (Michigan State University), Tony Capizzo and Jane Slater (USDA Agricultural Research Service) for assistance in the laboratory rearing the emerald ash borer and *Oobius agrili*, and field deployment and survey of emerald ash borer eggs. We are also grateful to Doug Luster and Roger Fuester (USDA Agricultural Research Service) for helpful comments to an early version of the manuscript.

References

Bauer, L.S., Liu, H.P., 2007. *Oobius agrili* (Hymentoptera: Encyrtidae), a solitary egg parasitoid of emerald ash borer from China. In: Mastro, V., Lance, D., Reardon, R., Parra, G. (comps), Proceedings Emerald Ash Borer and Asian Longhorned Beetle Research and Development Review Meeting, October 29–November 2, 2006, Cincinnati, OH. USDA Forest Service, FHTET 2007-04, pp. 63–64.

Bauer, L.S., Liu, H.P., Gao, R.T., Zhao, T.H., 2006. Egg and larval parasitoids of emerald ash borer from China: potential for biocontrol in North America. In: Mastro, V., Reardon, R., Parra, G. (comps.), Proceedings of the 2005 Emerald Ash Borer Research and Technology Development Meeting, September 26–27, 2005, Pittsburgh, PA. FHTET-2005-16. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV, pp. 48–49.

Bauer, L.S., Liu, H.P., Miller, D.L., Gould, J., 2008. Developing a biological control program for emerald ash borer (*Agrilus planipennis*), an invasive ash pest in North America. Michigan Entomological Society Newsletter 53, 38–39. <http://www.nrs.fs.fed.us/pubs/jrnl/2008/nrs_2008_bauer_002.pdf>.

Bauer, L.S., Liu, H.P., Miller, D.L., 2009. Emerald ash borer biological control: rearing, releasing, establishment, and efficacy of parasitoids. In: McManus, K., Gottschalk, K. (Eds.), Proceedings 20th U.S. Department of Agriculture Interagency Research Forum on Invasive Species, January 13–16, 2009, Annapolis, MD. USDA Forest Service, Gen. Tech. Rep. NRS-P-51, pp. 7–8.

Bauer, L.S., Gould, J., Duan, J.J., 2010. Can biological control of emerald ash borer save our ash? Michigan Entomological Society Newsletter 55, 26–27.

Bauer, L.S., Gould, J., Duan, J.J., Hansen, J.A., Cossé, A., Miller, D.L., Abell, K.J., Driesche, R., Lelito, J., Poland, T.M., 2011. Sampling methods for recovery of exotic emerald ash borer parasitoids after environmental release. In: McManus, K. A., Gottschalk, K.W., (Eds.), Proceedings 22nd U.S. Department of Agriculture interagency research forum on invasive species. January 12-15, 2011 Annapolis, MD. Gen. Tech. Rep. NRS-P-xx. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

Bell, P., 2005. National emerald ash borer management plan, p. 3. In: Mastro, V., Reardon, R. (Eds.), Emerald Ash Borer Research and Technology Development Meeting, Romulus, Michigan, October 5–6, 2004. USDA, Forest Service, FHTET-2004-15. 83p.

Canadian Food Inspection Agency, 2010. Emerald Ash Borer – *Agrilus planipennis*, published online at <http://www.inspection.gc.ca/english/plaveg/pestrava/agrpla/agrplae.shtml>.

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

CAS (Chinese Academy of Science), 1986. *Agrilus marcopoli* Obenberger. Agriculture Insects of China (part I). China Agriculture Press, Beijing, China, p. 445 (Translated by H.-P. Liu), posted at: <http://nrs.fs.fed.us/disturbance/invasive_species/eab/local-resources/downloads/biologyCAS1986.pdf>.

Duan, J.J., Fuester, R.W., Wildonger, J., Taylor, P.H., Barth, S., Spichiger, S.E., 2009. Parasitoids attacking the emerald ash borer (Coleoptera: Buprestidae in Western Pennsylvania. Florida Entomologist 92, 588–592.

Duan, J.J., Bauer, L.S., Ulyshen, M.D., Gould, J., Van Driesche, R., 2010a. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biological Control 39, 170–174.

Duan, J.J., Ulyshen, M.D., Bauer, L.S., Gould, J., Van Driesche, R., 2010b. Measuring the impact of biotic factors on populations of immature emerald ash borer (Coleoptera: Buprestidae). Environmental Entomology 39, 1513–1522.

Gould, J., Bauer, L.S., Duan, J.J., Fraser, I., Ulyshen, M., 2011. Recovery of parasitoids of the emerald ash borer, *Agrilus planipennise*. In: McManus, K., Gottschalk, K. (Eds.), Proceedings 22nd U.S. Department of Agriculture Interagency Research Forum on Invasive Species, January 12–15, 2011, Gen. Tech. Rep. NRS-P-xx. USDA Forest Service Northern Research Station, New Town Square, PA.

Haack, R.A., Jendek, E., Liu, H.P., Marchant, K.R., Petrice, T.R., Poland, T.M., Ye, H., 2002. The emerald ash borer: a new exotic pest in North America. Newsletter of the Michigan Entomological Society 47, 1–5.

Kovacs, F.K., Haight, R.G., McCullough, D.G., Mercader, R.J., Siegert, N.W., Leibhold, A.M., 2010. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. Ecological Economics 69, 569–578.

Liu, H.P., Bauer, L.S., 2007. Is biological control a management option for emerald ash borer in North America? In: Gottschalk, K. (Ed.), Proceedings, 17th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2006; Gen. Tech. Rep. NRS-P-10. U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA, p. 66.

Liu, H.P., Bauer, L.S., Gao, R.T., Zhao, T.H., Petrice, T.R., Haack, R.A., 2003. Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. The Great Lakes Entomologist 36, 191–204.

Liu, H.P., Bauer, L.S., Miller, D.L., Zhao, T.H., Gao, R.T., Song, L., Luan, Q., Jin, R., Gao, C., 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42, 61–71.

McCullough, D.G., Siegert, N.W., Bedford, J., 2009. Slowing ash mortality: a potential strategy to slam emerald ash borer in outlier sites. In: McManus, K., Gottschalk, K. (Eds.), Proceedings 20th U.S. Department of Agriculture Interagency Research Forum on Invasive Species, January 13–16, 2009, Annapolis, MD. Gen. Tech. Rep. NRS-P-51. USDA Forest Service Northern Research Station, New Town Square, PA, pp. 44–46.

Mercader, R.J., Siegert, N.W., Liebhold, A.M., McCullough, D.G., 2011. Simulating the effectiveness of three potential management options to slow the spread of emerald ash borer (*Agrilus planipennis*) populations in localized outlier sites. Canadian Journal of Forest Research 41, 254–264.

Michigan State University, 2010. Emerald ash borer information, published online at <http://emeraldashborer.info/>.

Nowak, D., Crane, D., Stevens, J., Walton, J., 2003. Potential damage from emerald ash borer. <http://nrs.fs.fed.us/disturbance/invasive_species/eab/local-resources/downloads/EAB_potential.pdf>.

Poland, T.M., McCullough, D.G., 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104, 118–124.

Poland, T.M., McCullough, D.G., Herms, D.A., Bauer, L.S., Gould, J.R., Tluczek, A.R., 2010. Management Tactics for emerald ash borer: chemical and biological control. In: McManus, K., Gottschalk, K. (Eds.), Proceedings 21th U.S. Department of Agriculture Interagency Research Forum on Invasive Species, January 12–15, 2010, Annapolis, MD. Gen. Tech. Rep. NRS-P-75. USDA Forest Service Northern Research Station, New Town Square, PA, pp. 46–49.

SAS Institute Inc., 2008. JMP® 8 Introductory Guide. SAS Institute Inc, SAS Campus Drive, Gary, NC 2751, USA.

USDA APHIS, 2003. Emerald ash borer: quarantine and regulations. 7 CFR Part 301 [Docket No. 02-125-1] Federal Register 68, 59083–59092.

USDA APHIS, 2007. The proposed release of three parasitoids for the biological control of the emerald ash borer (*Agrilus planipennis*) in the continental United States: environmental assessment. Federal Register 72, 28947–28948 (Docket No. APHIS-2007-0060).

Van Driesche, R.G., Idoine, K., Rose, M., Bryan, M., 1998. Release, establishment and spread of Asian natural enemies of euonymus scale (Homoptera: Diaspididae) in New England. Florida Entomologist 81, 1–9.

Wei, X., Wu, Y., Reardon, R., Sun, T.H., Lu, M., Sun, J.H., 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* Fairmaire) in China. Insect Science 14, 367–373.

Yang, Z.-Q., Strazanac, J.S., Marsh, P.M., Van Achterberg, C., Choi, W.-Y., 2005. First recorded parasitoid from China of *Agrilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 98, 636–642.

Yang, Z.-Q., Srazanac, J.S., Yao, Y.-X., Wang, X.-Y., 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). Proceedings of the Entomological Society of Washington 108, 550–558.

Zhang, Y.Z., Huang, D.W., Uang, D.W., Zhao, T.H., Liu, H.P., Bauer, L.S., 2005. Two new species of egg parasitoids (Hymenoptera: Encyrtidae) of wood-boring beetle pests from China. Phytoparasitica 33, 253–260.