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Long-term monitoring of the introduced emerald ash borer (Coleoptera: Buprestidae) egg parasitoid, *Oobius agrili* (Hymenoptera: Encyrtidae), in Michigan, USA and evaluation of a newly developed monitoring technique

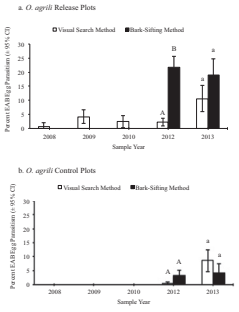
Kristopher J. Abell [a,c,*], Leah S. Bauer [b,c], Jian J. Duan [d], Roy Van Driesche [a]

[a] Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003, 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] USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 19713, United States

HIGHLIGHTS

- *O. agrili* has established in Michigan, USA.
- *O. agrili* parasitism has increased since being introduced.
- Bark sifting is an effective method of estimating *O. agrili* parasitism.

GRAPHICAL ABSTRACT



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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest of ash trees (*Fraxinus* spp.) in North America from China. The egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) was introduced from China as a biological control agent for this pest in Michigan and throughout the infested area of the United States. A critical component of any biological control program is post-release monitoring and evaluation; however, because of the small size and cryptic nature of *O. agrili*, evaluation of its impact is difficult. We compared two methods for measuring parasitism of emerald ash borer eggs: (1) timed visual searches of bark on standing ash trees and (2) bark collection, sifting, and sorting. Both methods were carried out in paired parasitoid-release and control plots, the visual search method over a six-year period (2008–2013) and the more recently developed bark-collection and sifting method for 2 years (2012–2013). The visual search method found parasitism in release plots remained low (0.7–4.2%) in samples taken from 2008 to 2012 and reached 10.6% in 2013. In comparison, the bark-sifting method found that rates of egg parasitism were considerably higher in release plots, 21.8% and 18.9% for samples taken in 2012 and 2013, respectively. These findings indicate that the population-level impact of *O. agrili* is increasing and may be an important source of mortality for EAB populations. We recommend the bark-collection and sifting method as the more effective method to recover parasitoids and estimate parasitism rates of *O. agrili*.

* Corresponding author at: Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003, United States.
E-mail address: abellk@msu.edu (K.J. Abell).

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1. Introduction

Since its accidental introduction to North America from China in the 1990s, emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) (Coleop.: Buprestidae) has killed tens of millions of ash (*Fraxinus* spp.) trees (Haack et al., 2002; Cappaert et al., 2005; Poland and McCullough, 2006; Bray et al., 2011; Herms and McCullough, 2014). Although the impact of losing ash in forested ecosystems is unknown, Kovacs et al. (2010) estimates > 1 billion US dollars per year will be expended for removal and replacement or treatment of dead ash trees on developed lands. In addition, a number of ash-specialist insects, particularly Lepidoptera, may be threatened by the loss of ash trees (Wagner, 2007). Eradication efforts directed against EAB were expensive and ineffective due to human transport of infested ash materials and natural dispersal of EAB adults which can exceed 7 km/day (McCullough et al., 2005; GAO (Government Accounting Office), 2006; Taylor et al., 2010). Moreover, detection methods cannot detect new infestations until several years after beetle arrival (Poland and McCullough, 2006). Insecticidal treatment may be effective (Herms et al., 2009), but is impractical, costly, and environmentally prohibitive over large areas. At the landscape level, protection of ash in forests will require enhanced tree resistance to EAB and higher levels of mortality from natural enemies, the latter achieved through importation of specialized natural enemies from the borer's native range. Three parasitoid species discovered in China (part of EAB's native range) (Liu et al., 2003, 2007) were imported and released in Michigan and other parts of North America as part of a biological control program against EAB (Bauer et al., 2008). These introduced species are two larval parasitoid species – *Tetrastichus planipennisi* Yang (Eulophidae) (Yang et al., 2005) and *Spathius agrili* Yang (Braconidae) (Yang et al., 2006) – and one egg parasitoid species, *Oobius agrili* Zhang and Huang (Encyrtidae) (Zhang et al., 2005).

Several studies have monitored the establishment and evaluated the effectiveness of introduced larval parasitoids of EAB (Duan et al., 2012a, 2013, 2014), but relatively few studies have measured the impacts of *O. agrili*, largely because EAB eggs are concealed in bark crevices and are difficult to find. Several sampling methods have been used to estimate rates of egg parasitism, including (1) the use of sentinel eggs (placing them under flaps of bark), (2) timed visual searches for naturally occurring EAB eggs on ash trees, (3) deployment of sentinel egg logs (short lengths of ash bearing EAB eggs) (Duan et al., 2011, 2012b), and (4) holding ash logs or bark samples in emergence tubes for parasitoid emergence (Bauer et al., 2011, 2012). Previous studies focused primarily on developing methods to detect and recover *O. agrili* rather than measuring population-level parasitism rates. This study presents estimates of population-level EAB egg parasitism in long-term study plots in Michigan and compares rates of parasitism from *O. agrili* in timed, field searches for EAB eggs versus a new method we developed for the recovery of EAB eggs from bark samples returned to the laboratory for sifting, sorting, and examination.

2. Methods

2.1. Study sites and O. agrili releases

In 2008, observations of egg parasitism were made at three forested sites in Ingham County, MI: Central/Nancy Moore Park (42° 43′N, 84° 25′W), Legg Park/Harris Nature Center (42° 41′N, 84° 22′W), and Burchfield Park (42° 34′N, 84° 36′W). In 2011, three additional sites were added to the study, two in Gratiot County, MI: Gratiot-Saginaw State Game Area (43° 23′N, 84° 45′W), and Maple River State Game Area (43° 08′N, 84° 32′W) and one in Shiawassee County, MI: Rose Lake State Wildlife Area (42° 48′N, 84° 20′W). For a detailed map of EAB biocontrol study sites in Michigan see Duan et al. (2013) or MapBiocontrol (2014). Two plots were established at each site, one a parasitoid release plot and the other a control plot. The release and control plots for each site are approximately 0.8 km apart, except for the Maple River State Game Area plots, which are 9.5 km apart. The relatively short distance between release and control plots was chosen to minimize environmental variability and to monitor establishment in adjacent areas where releases had not been made. Sites had a mixture of green ash (*Fraxinus pennsylvanica* Marshal) and white ash (*Fraxinus Americana* Linnaeus).

Laboratory cultures of *O. agrili*, originating from EAB eggs sampled near Changchun in Jilin Province, China from 2005 to 2009, are maintained in the USDA Forest Service Northern Research Station Laboratory in East Lansing, MI and mass-reared as EAB biocontrol agents by the USDA APHIS EAB Biocontrol Facility in Brighton, MI (Zhang et al., 2005; Bauer and Liu, 2006; Liu et al., 2007; Bauer et al., 2012). As a parthenogenic species, only females are used for colony maintenance and environmental release. Three- to five-day-old adult female *O. agrili* were released from clear plastic cups (355 ml) streaked with honey (10–12 adults/cup) onto the trunks of EAB-infested ash trees at each release plot (Duan et al., 2012a,b). The release history for these study sites are reported in Table 1.

2.2. Timed visual egg searches

To estimate parasitism by *O. agrili*, visual inspections for EAB eggs on the bark of ash trees were conducted between April and August (each year from 2008 to 2013 except 2011) (Table 1). All references to year in this study refer to the year the sample was collected, but it should be noted that the majority of parasitism occurred in previous years. Searches were made on the trunk (up to 2 m high) by carefully examining the surface of the bark of live, EAB-infested ash trees in the field, removing small bark pieces with a utility knife to expose EAB eggs concealed between layers of bark and in cracks and crevices. Each ash tree was searched by one or two people for a total of 30 min, and all eggs found were collected and returned to the laboratory where they were examined microscopically for signs of *O. agrili* parasitism. Healthy EAB eggs typically turn brown when mature or hatched. Eggs parasitized by *O. agrili* typically turn black, although some are brown and not all black eggs are parasitized. Therefore all unhatched eggs were dissected for confirmation of *O. agrili* by observing a larva, pupa, or unemerged adult. Eggs were also categorized as parasitized by the presence of an adult *O. agrili*-emergence hole, which is round, on the dorsal surface of the egg, and dark droplets of meconium are often visible on the interior surface. In contrast, the emergence hole of an EAB larva from a hatched egg is ovoid, on the ventral surface of the egg, and filled with light-colored frass pellets. Using these criteria, eggs showing the signs and symptoms of parasitism by *O. agrili* were categorized as parasitized. In 2008, 20 trees were searched in each plot. In 2009, 2010, and 2013, 10 trees were searched in each plot (except Rose Lake control where 6 trees were examined in 2013). In 2011, searches were not made until the fall and subsequently were combined with searches made in the spring of 2012 because biologically both sampling periods reflected parasitism from 2011. For this sampling period, 8 trees were searched at the Maple River State Game Area control plot, and 10–12 trees were searched at each of the other plots. For data analyses, egg counts were pooled across all sites by treatment (release vs. control) and percent parasitism was calculated by dividing the total number of parasitized eggs by the total number of eggs found (parasitized and unparasitized).

Table 1
Release history of *O. agrili* and chronology of timed visual search and bark-sifting methods in study sites.

Site	Year	*O. agrili* released		Month of recovery methods		
		Month(s)	Total N	Month(s)	Visual	Sift
Central park	2007	Aug	700	–	–	–
	2008	June–Aug	330	July/Aug	×	–
	2009	June	300	July	×	–
	2010	–	–	July	×	–
	2011	–	–	Oct	×	–
	2012	–	–	May	×	×
	2013	–	–	Apr–May	×	×
Legg park	2008	July	200	July/Aug	×	–
	2009	June	300	July	×	–
	2010	–	–	July	×	–
	2011	–	–	Oct	×	–
	2012	–	–	May	×	×
	2013	–	–	Apr–May	×	×
Burchfield park	2008	July–Aug	200	July/Aug	×	–
	2009	June	300	July	×	–
	2010	–	–	July	×	–
	2011	–	–	Oct	×	–
	2012	–	–	Apr–May	×	×
	2013	–	–	Apr–May	×	×
Gratiot saginaw	2009	Aug	375	–	–	–
	2010	June–July	1110	–	–	–
	2011			Nov	×	–
	2012			May	×	x
	2013			Apr–May	×	×
Maple river	2010	June–July	1165	–	–	–
	2011			Oct	×	–
	2012			May	×	×
	2013			Apr–May	×	×
Rose lake	2010	July	1160	–		–
	2011			Nov	×	–
	2012			May	×	×
	2012			Apr–May	×	×

2.3. Bark sampling and sifting

2.3.1. General sampling method

A fixed area of bark (10 × 100 cm in May 2012 and 10 × 50 cm in May 2013) was sampled before *O. agrili* emergence, providing an estimate of egg parasitism during 2011 and 2012. The bark was sampled from the same 10 trees used for the timed visual egg searches described previously, except that in 2012 only five trees were sampled per plot and no samples were collected from control plots at Maple River Game Area or Rose Lake Wildlife Area. In addition, some trees were so extensively sampled (as evidenced by the scaled look of the trunk from bark flake removal) during the visual surveys that nearby trees were sampled in their place. Bark samples were taken from the first meter of the trunk of the tree. In 2012, two bark samples were taken from each tree, one from the south facing and one from the west facing side of the tree. The south and west sides were chosen because of anecdotal observation from debarking trees that more EAB larvae were on the south and west facing sides, most likely because these aspects of the tree receive the most sunlight. Both the south- and west-side samples were monitored for emergence (using emergence tubes as described below), but only the west side sample was subjected to the bark-sifting method described below. In 2013, only one bark sample was taken from each tree on the southwest facing side and the area of bark was decreased because we determined a smaller amount of bark was sufficient to recover an adequate number of EAB eggs, leaving more bark to sample in subsequent years.

To collect samples of outer bark, a sheet of plastic was tightly wrapped around the base of the tree with duct tape, and the edges held up in the shape of a cone around the tree. The area of bark was measured, delineated, and sheared off with a drawknife into the inverted cone of plastic sheeting. The bark sample was then funneled into a paper bag, labeled, and taken back to the laboratory, where each bark sample was placed into an emergence tube. Emergence tubes consisted of a cylindrical mailing tube with one end capped and with a clear inverted funnel and collection cup attached to the other end. After 10 weeks, the collection cup for each sample was checked for emerged *O. agrili* adults. Recovery of *O. agrili* from emergence tubes was used solely to document the parasitoid's presence or absence in each study plot before comparing the visual search and bark-sifting methods. Following examination of emergence tube collection cups, the bark was removed from each emergence tube and sifted with standard window screen (1.2 × 1.2 mm opening, EAB eggs are approximately 1 × 0.75 mm) into a white ceramic baking dish. Under a dissection microscope, EAB eggs were then sorted from the other fine debris that passed through the screening. Eggs were categorized as either parasitized or unparasitized using the same criteria as described above for the timed visual egg search method. During this process, several bark samples for the 2011 egg-parasitism estimates were lost (one from the Central Park release and control plot, three from the Legg Park release plot, one from the Legg Park control plot). Six trees were sampled from Rose Lake Wildlife Area release plot for the 2012 egg-parasitism estimates because these were the only remaining live ash that could be found. Percent parasitism was calculated by dividing the total number of parasitized eggs by the total number of eggs (parasitized plus unparasitized), as above for timed visual egg searches.

2.3.2. Test of parasitism in sifted vs. total bark material

Bark samples were not processed in any way prior to sifting, except to cut pieces longer than 20 cm so that they would fit in

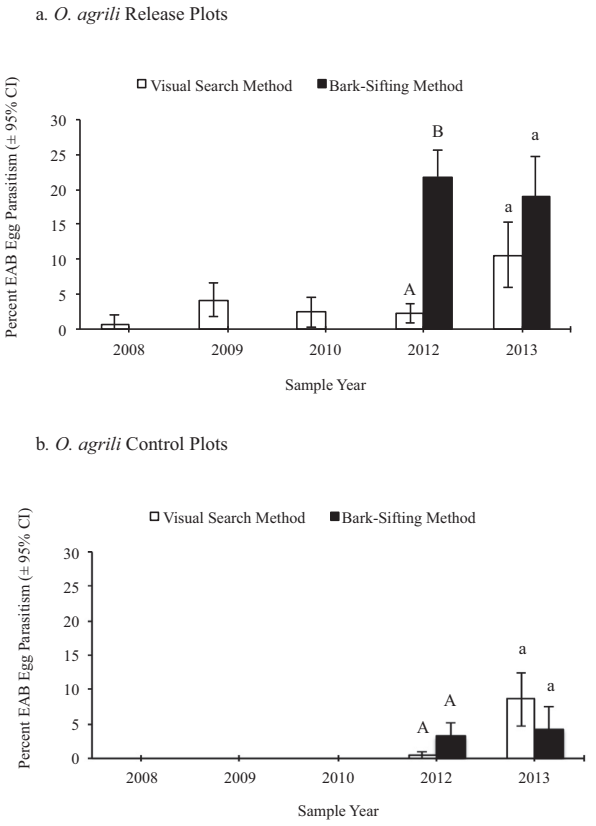

Fig. 1. Percent EAB egg parasitism (pooled ± 95% CI) using the timed visual search (2008–2013) and bark-sifting (2012–2013) methods for sampling EAB eggs and parasitism by *O. agrili* in (a) release and (b) control plots following *O. agrili* introductions (2007–2010). Year on the *x*-axis is the year samples were collected and represents percent parasitism that occurred primarily in the previous year. Samples collected in 2011 were taken in the fall and therefore combined with samples collected in spring 2012. Percent parasitism was calculated by dividing the total number of parasitized eggs by the total number of eggs (parasitized and unparasitized), pooled across plot type. Likelihood chi-square tests based on a logistical regression model were used to compare the visual egg search and bark-sifting methods for each treatment and year separately. Letters indicate significant differences between methods for each year (uppercase letters for differences in 2012 and lowercase for differences in 2013).

the screening. Because the majority of the bark did not pass through window screening (~99%), we ran a side test to determine if omitting this material from examination biased % parasitism values. To test that proposition, all of the bark that did not pass through the screen from 12 samples was dissected and examined for additional EAB eggs. Bark was dissected by carefully examining the surface of the bark and removing small bark pieces with a utility knife to expose EAB eggs concealed between layers of bark and in cracks and crevices. Rates of egg parasitism were compared between eggs collected from sifting vs. eggs dissected from larger bark samples. This comparative test was done using May 2012 samples from both release and control plots.

2.3.3. Test of aspect on bark collection and sifting method

As described in Section 2.3.1, bark samples taken for the bark-sifting method were collected from the southwest aspect of each tree because of the observation that there tends to be more EAB larva on this side of the tree, probably because this aspect receives greater radiant heat from the sun. Thus, bark samples taken from the southwest aspect should have more eggs thereby maximizing egg sample size. To test this hypothesis and to determine if samples taken from a particular aspect introduces a bias in estimating parasitism rates (due to density dependent parasitism or other unknown factors), bark was taken, separately, from both the southwest and northeast facing sides of the 10 sample trees at two release plots (Central Park and Burchfield Park) in May 2013, and processed to determine rates of parasitism in the eggs detected from each aspect.

2.4. Statistical analysis

Logistic regression was used to compare parasitism rates and percentage of trees with parasitized eggs between the two sampling methods (visual search and bark-sifting). First, a model to examine all parameters and sampling method interactions was used: site, treatment (control/release plots), sampling method (visual and bark-sifting), year, method * year, method * treatment, and method * site. When method * site was not significant, data were pooled across sites for each treatment. Likelihood chi-square tests based on a logistical regression model were then used to compare the visual egg search and bark-sifting methods for each treatment and year separately. Ash trees sampled in each plot were considered the sampling units. Logistic regression was also used to test for a difference in parasitism rate using the bark-sifting method between bark samples collected on the southwest aspect vs. the northeast aspect of ash trees. All statistical analyses were performed using JMP 11.1.1.

3. Results

3.1. Timed visual egg search

Each year in both release and control plots, EAB eggs remained common, with 58–92% of sampled trees bearing EAB eggs. This demonstrated both that host eggs were widely present spatially (tree to tree) within plots and of similar abundance (as % trees bearing eggs) from year to year. These data suggest that host presence on sampled trees did not change strongly over the sampling period in ways that might have affected parasitism rates (such as if hosts become scarce late in the study).

In release plots, *O. agrili* parasitism varied from 0.7% (1/140) to 4.2% (11/261) in samples from 2008 to 2012 and was highest (10.6%, [18/170]) in samples from 2013 (Fig. 1a). In control plots, parasitism was zero or minimal (0.4% [2/495]) in samples from 2008 to 2012, but was similar (8.6% [17/198]) to that in release plots in samples from 2013 (Fig. 1b). The Burchfield release plot was the only one in which parasitism was detected in the year immediately following *O. agrili* release (2009 and 2010) (Fig. 2). Parasitism was detected in Central and Legg release plots in 2012, 2–3 years after the first releases of *O. agrili*. Likewise, parasitism in the Gratiot-Saginaw and Maple River release plots was first detected two years after the first *O. agrili* release despite having a greater number of *O. agrili* released. Only the Burchfield release plot detected an increase in parasitism over time (the increase seen in Legg Park is based on a sample of only seven eggs and is therefore likely to be an inaccurate estimate). Burchfield and Legg Park were the only control plots where parasitism was detected, three years after the first introduction of *O. agrili* into release plots.

In addition to the percentage of eggs parasitized, two other measures reflect the gradual increase in the parasitoid's density over time: the percentage of release plots from which the parasitoid was recovered, and the percentage of sampled trees within plots from which parasitized eggs were recovered. From 2008 to 2010, *O. agrili* parasitism was detected in 44% of all release plot × year samples (4 recoveries/9 release plot–year combinations), which increased in the 2012–2013 to 66% (8 plots with recoveries/12 plot–year combinations). For control plots, no recoveries of parasitoids were made in the first period (0 recoveries/9 plot–year

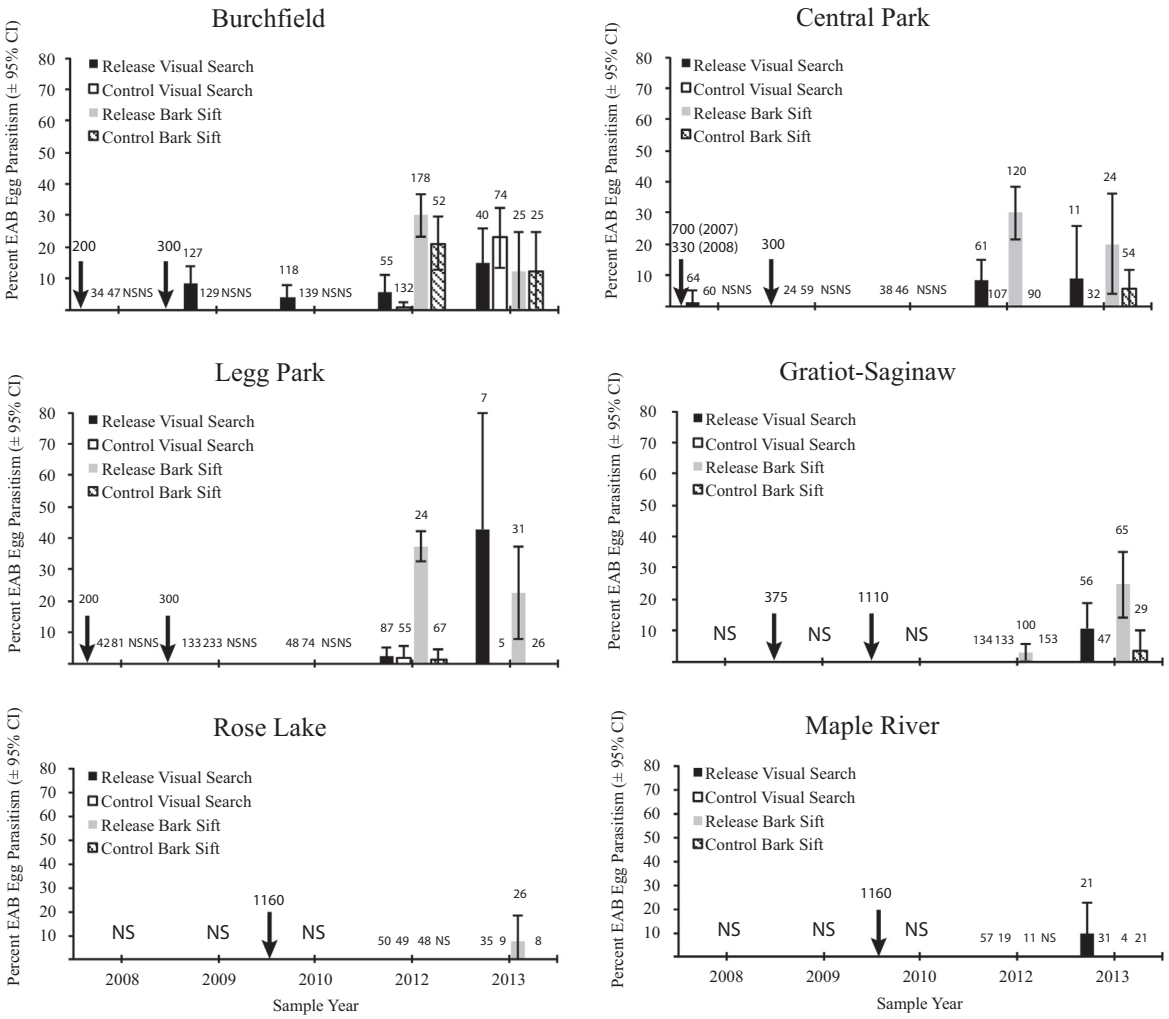

Fig. 2. Percent EAB egg parasitism (pooled ± 95% CI) by *O. agrili* in each site (control and release plots) using the timed visual search and bark sifting methods. Year on the *x*-axis is the year samples were collected and represents parasitism that occurred primarily in the previous year. Samples collected in 2011 were taken in the fall and therefore combined with samples collected in spring 2012. Percent parasitism was calculated by dividing the total number of parasitized eggs by the total number of eggs (parasitized and unparasitized), pooled across sampled trees in each plot. Arrows indicate when *O. agrili* releases were made in each release plot, and numbers over arrows indicate the number of *O. agrili* released. "NS" indicates no sample was taken in sites for a given year. Numbers over bars indicate the number of EAB eggs (parasitized and unparasitized) sampled in each plot.

combinations), and in the second period [2012–2013]) only 33% of control plots had parasitoids (4 recoveries/12 plot–year combinations). In terms of the percentage of visually searched trees on which parasitism was detected, the rate of parasitoid detection per tree rose in release plots from 6.7% (8/120 trees) in 2008–2010 to 12.7% (16/126 trees) in 2012–2013 (Fig. 3a). In control plots, the percentage of trees with visually detected parasitized eggs was 0% (0/120 trees) in 2008–2010 and rose to 5.6% (6/108 trees) in 2012–2013 (Fig. 3b).

3.2. Bark collection and sifting

Using the bark-sifting method pooled over all sites, estimates of egg parasitism were 21.8% (102/468 eggs) for 2012 and 18.9% (33/175) for 2013 in release plots (Fig. 1a), and 3.3% (12/363 eggs) for 2012 and 4.3% (7/163) for 2013 in control plots (Fig. 1b). There was a significant method * year and method * plot type interaction ($\chi^2 = 24.3348$, df = 1, $P < 0.0001$ and $\chi^2 = 5.3096$, df = 1, $P = 0.0212$). Therefore, sites were pooled across year and treatment and sampling method was compared for each year and plot type combination using a Bonferroni corrected P value of 0.0125. The rate of parasitism in release plots detected by bark-sifting was significantly greater in 2012 than that detected by visual search (Likelihood ratio $\chi^2 = 93.023$, df = 1, $P < 0.0001$), but not in 2013 (Likelihood ratio $\chi^2 = 4.745$, df = 1, $P = 0.0294$) (Fig. 1a). The rate of parasitism detected in control plots was significantly greater in 2012 using the bark-sifting method compared to the visual search (Likelihood ratio $\chi^2 = 11.57$, df = 1, $P = 0.0007$), but not in 2013 (Likelihood ratio $\chi^2 = 2.754$, df = 1, $P = 0.0970$) (Fig. 1b).

The percentage of plots where *O. agrili* was recovered in 2012 and 2013 combined was greater for bark-sifting compared to visual search for both plot types: 75% (9 recoveries/12 plot–year combinations) vs. 67% (8 out of 12) for release plots and 50% (5 out of 10) vs. 25% (3 out of 12) for control plots (Fig. 2). Rearing of *O. agrili* from bark samples in emergence tubes gave a lower estimate of the percentage of plots with the parasitoid than did the other two methods for release plots, i.e., 42% (5 out of 12), and was intermediate with other methods in control plots, i.e., 33% (4 out of 12).

The percentage of sampled trees on which *O. agrili* parasitism was detected using the bark-sifting method was 27.9% (24/86 trees) in release plots (Fig. 3a) and 11.1% (8/72 trees) in control plots (Fig. 3b) in 2012 and 2013 combined. Site * method interaction was not significant ($\chi^2 = 4.1849$, df = 1, $P = 0.0408$), so sites were pooled and sampling method was compared for each year

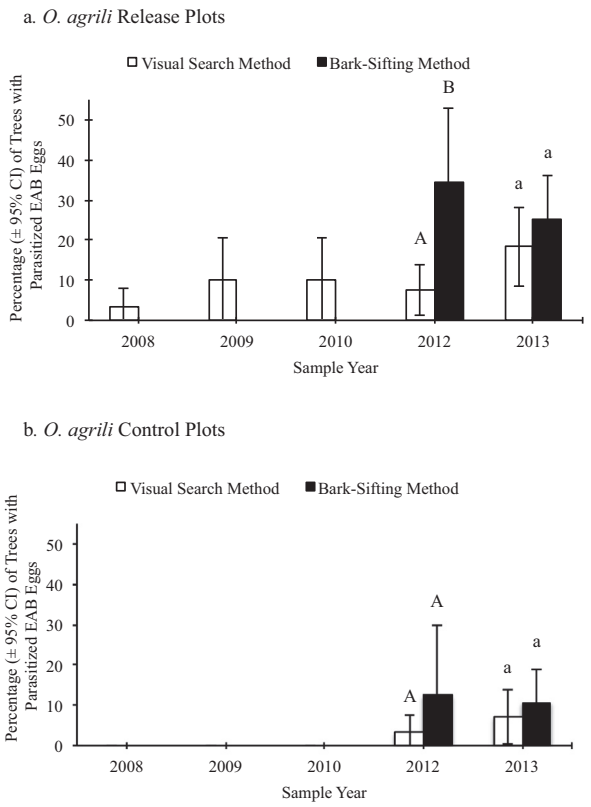

Fig. 3. Percent of sampled ash trees (pooled ± 95% CI) on which *O. agrili* parasitized eggs were found using the timed visual search (2008–2013) and bark sifting (2012–2013) methods in (a) release and (b) control plots following *O. agrili* introductions (2007–2010). Year on the *x*-axis is the year samples were collected and represents parasitism that occurred primarily in the previous year. Samples collected in 2011 were taken in the fall and therefore combined with samples collected in spring 2012. Percentage of trees with parasitized eggs was calculated by dividing the total number of trees with parasitized eggs by the total number of trees sampled, pooled across plot type. Likelihood chi-square tests based on a logistical regression model were used to compare the visual egg search and bark-sifting methods for each treatment and year separately. Letters indicate significant differences between methods for each year (uppercase letters for differences in 2012 and lowercase for differences in 2013).

and plot type combination. The percentage of sampled trees with *O. agrili* parasitized eggs using the bark-sifting method was significantly greater compared to visual searching in release plots in 2012 (Likelihood ratio $\chi^2 = 9.5177$, df = 1, $P = 0.0020$) but not in 2013 (Fig. 3a).

3.3. Test of parasitism in sifted vs. total bark material

On average, dissecting bark after sifting found 10 eggs and 0.75 parasitized eggs. This resulted in an overall mean difference in parasitism estimates of only 2.8 percentage points (23.1% [total sample] vs. 25.9% [sifted material only]) between just sifting bark and sifting plus dissecting bark.

3.4. Test of tree aspect on bark collection and screening method

When pooled over the two plots where samples for this test were taken, percent parasitism was not significantly different between samples taken from the southwest aspect compared to samples taken from the northeast aspect at an alpha level of 0.05 (16.3% vs. 6.7%, $\chi^2 = 1.8261$, df = 1, $P = 0.1766$).

4. Discussion

Our results are the first to report the level of effectiveness of *O. agrili* at parasitizing EAB eggs over an extended period (up to five years after their environmental release). Bark-sifting was more effective than visual searching, both at estimating the rate of parasitism and at determining the percentage of trees in a stand where parasitized eggs occurred but not in all years.

The visual search method had several logical disadvantages. First, visual searches are done in the field where the observer is subject to variations in light and weather conditions. Second, eggs are small and difficult to find even with the aid of a magnifying lens. Third, the process of removing small pieces of bark to search for eggs likely sometimes results in the loss of eggs with the bark that is removed. Fourth, visual searches are subject to variation in the ability of the observer. Bark sampling and sifting method eliminates these disadvantages, but is more time consuming.

Both methods share the disadvantage that it is impossible to differentiate between eggs from the current generation and eggs from previous generations making it difficult to detect changes in estimated parasitism rates from year to year. This may be why parasitism rates seen in this study were virtually the same in 2012 and 2013. Alternatively, *O. agrili* populations may simply not have measurably increased in this time period.

Overall, parasitism rates were low (less than 11%) using the visual search method and it was not until 2013, 5 years after *O. agrili* releases began, that parasitism rates rose above 2.4%. In contrast, the bark-sifting method estimated much higher parasitism rates than the visual search method, nearly 10 times greater at release sites the first year and nearly 2 times greater the following year. In control sites, the bark-sifting method estimated about 8 times greater parasitism the first year, but about half as much the following year.

Bark-sifting estimates of egg parasitism rates were virtually the same from year to year in control and release plots, respectively, while visual search method estimates were variable. This would seem to corroborate the disadvantages of the visual search method described earlier, and suggests that this method provides a less accurate estimate of percent parasitism of EAB eggs. The bark-sifting method on the other hand provided consistent estimates from year to year and generally has fewer disadvantages (as described above). Based on our findings, we recommend the bark-sifting method to estimate percent parasitism, and the visual search method for *O. agrili* detection only.

How rates of parasitism in the U.S. EAB-invaded area compare to rates of parasitism in Chinese native range is a key measure of success of the biological control program. Using a visual bark searching method similar to the one used in this study, Liu et al. found an average parasitism rate of 21.8% in China in 2005 (Liu et al., 2007). In comparison, using the same sampling method, we found parasitism reached about 10% within 5 years of release, about half the parasitism rate found in China. This suggests that parasitism rates in the United States might reach equivalent levels to those in the native range over the next several years. It is interesting to note that the bark-sifting method suggests that real parasitism rates are actually higher that those determined by visual egg searches, suggesting that *O. agrili* might have an even greater impact as a biological control agent of EAB than supposed based on visual search parasitism estimates from China.

Our findings indicate that *O. agrili* has established in all but one of the sites where it was released. It is possible that overall parasitism was overestimated to some degree in our study since parasitism was lower when bark samples were taken from the northeast side of the tree compared to the southwest side of the tree. However, EAB are unequally distributed as well, being most abundant

on south and west faces of ash trees. To improve this method for future monitoring of *O. agrili*, we recommend taking an area of bark around the entire circumference of each tree. This should eliminate any bias of egg density related to tree aspect due to differential thermal energy from the sun and associated density dependent parasitism.

While we did show that *O. agrili* has successfully dispersed from release to control plots, we found parasitized eggs on, at most, only 35% of trees in either control or release plots, but host eggs were found on the majority of trees sampled (58–92%) for both the visual survey and bark-sifting methods. This suggests that *O. agrili* tend to be unevenly distributed and has not yet reached the same level of site saturation as *T. planipennisi*, which now occurs on 92% of trees in our release plots (Duan et al., 2013). This apparent unevenness has implications in regard to monitoring efforts and potential effectiveness of *O. agrili*. In the laboratory, *O. agrili* is primarily observed walking and makes only occasional short jumps or flights (Bauer, personal communication). If this behavior is consistent in the field, then *O. agrili* likely disperses slowly and irregularly. In the future, study of *O. agrili* dispersal and host search behavior could be extremely useful for further evaluating and predicting the effectiveness of *O. agrili* as a biological control agent of EAB. Moreover, these results support introductions of *O. agrili* over a wider geographic area and perhaps in larger numbers than used in this study.

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