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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)

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ABSTRACT

A field study was conducted in forested plots near Lansing, Michigan in 2008 and 2009 to evaluate the newly introduced egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) for control of the invasive emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). To measure parasitism by *O. agrili*, laboratory-reared "sentinel EAB eggs" were deployed under bark flaps on trunks of selected ash trees in both parasitoid-release and non-release control plots. In addition, naturally occurring EAB eggs were collected in both parasitoid-release and control plots to measure parasitism. While no parasitism was detected with either sentinel or naturally occurring EAB eggs in control plots in either 2008 or 2009, a low level of parasitism by *O. agrili* was detected in the parasitoid-release plots in both artificially deployed sentinel eggs (⩽1%) and field-collected, naturally occurring eggs (1.1–4.2%) in both years. In addition to losses due to parasitism by *O. agrili*, a large proportion (37–52%) of the field-deployed sentinel eggs disappeared, possibly due to predators such as ants, in both parasitoid-release and control plots. While no statistical differences in parasitism by *O. agrili* were detected between parasitoid release and control plots, other sources of egg mortality such as disappearance due to predation on eggs, varied significantly across study sites in both 2008 and 2009. The relevance of these findings to future release and evaluation strategies for *O. agrili* for biological control of the invasive emerald ash borer in the US is discussed.

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1. Introduction

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a serious invasive pest that has killed tens of millions of ash (*Fraxinus* spp.) trees in urban and forested areas of eastern North America since its introduction in the late 1990s (Haack et al., 2002). EAB has invaded 15 states (Illinois, Indiana, Iowa, Kentucky, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Tennessee, Virginia, West Virginia, and Wisconsin) (Michigan State University, 2010) and two Canadian provinces (Ontario and Quebec) (Canadian Food Inspection Agency, 2010). If EAB spreads throughout the range of ash in North America, over the next ten years, it will kill many times more ash trees, and cost over $1 billion per year just for treatment, removal and replacement of infested landscape ash trees within affected communities (Kovacs et al., 2010). Regulatory efforts to contain the pest via early detection, quarantine, and removal of infested ash trees have had little success (Cappaert et al., 2005). Moreover, chemical control cannot be used to protect native ash species in forest ecosystems because of prohibitive cost, general impracticality, and environmental hazards (Poland and McCullough, 2006). In contrast, biological control using insect parasitoids may have the potential to be a cost-effective, sustainable, and environmentally safe alternative.

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a solitary idiobiont parasitoid of EAB eggs, which are laid in bark crevices or under loose bark flakes of ash limbs or trunks. *Oobius agrili* reproduces via parthenogenesis; the female to male ratio is approximately 15:1 (Zhang et al., 2005; Bauer and Liu, 2006). Field studies in China, within the native range of EAB, show that *O. agrili* completes at least two generations per year, with parasitism peaking during July (56.3%) and August (61.5%), suggesting that this parasitoid be an important mortality factor affecting EAB populations (Bauer and Liu, 2006; Liu et al., 2007). *Oobius agrili* is one of three species of hymenopteran parasitoids from northern China

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that have been introduced for the biological control of EAB since 2007 (USDA APHIS, 2007; Bauer et al., 2008, 2009). Also introduced were *Spathius agrili* Yang (Braconidae), a gregarious larval ectoparasitoid (Liu et al., 2003; Yang et al., 2005) and *Tetrastichus planipennisi* Yang (Eulophidae), a gregarious larval endoparasitoid (Liu et al., 2003; Yang et al., 2006). As the only egg parasitoid, *O. agrili* is a particularly important component of the EAB biological control program because it poses no risk of interference with other parasitoids (native or introduced). Field surveys for EAB natural enemies in Michigan and Pennsylvania have not found parasitoids attacking EAB eggs (Liu and Bauer, 2007; Duan et al. 2009, 2010). With field releases underway in several states, methods to evaluate the field performance of *O. agrili* are clearly needed.

Few methods are available to detect and measure the impacts of egg parasitoids of wood-boring buprestid beetles, which lay eggs individually or in groups in concealed habitats such as beneath the bark flakes or in bark crevices of host trees. Because naturally occurring EAB eggs are most often found in such concealed sites, sampling them in the field (e.g., Liu et al., 2007) to detect parasitism or predation is time-consuming and labor intensive, with a high risk of overlooking or destroying eggs in the process. In the present study, we present a method whereby laboratory-reared "sentinel eggs" can be placed under bark flaps to measure the impact of *O. agrili* on survivorship of EAB eggs. In addition, we compare the efficacy of this method to that of sampling naturally occurring eggs, as developed by Liu et al. (2007).

2. Materials and methods

2.1. Study site

The study was conducted at four forested sites in Ingham County near Lansing, MI in 2008 and 2009. Site one (42°43′N/84°25′W) included two continuous Meridian Township, MI parks (Central and Nancy Moore Parks). Site two (42°41′N/84°22′W) spanned two other Meridian Township parks (Harris Nature Center and Legg Park). The third site (42°42′N/84°24′W), a Michigan State University research area (Dobie Reserve) also in Meridian Township, was located approximately 2 km from site one and 2.5 km from site two. The fourth site (42°34′N/84°36′W) was located in Holt, MI (≈25 km south of Lansing) in an Ingham County Park (William M. Burchfield Park), approximately 32 km away from the Meridian Township study sites. While all four sites were used in 2008, Dobie Reserve was excluded in 2009 due to high ash mortality in the stand.

At each site, two forested plots, separated from each other by 0.5–1 km, were selected and randomly assigned as either the parasitoid-release or the control plot. All study plots were stands of mixed deciduous forest with ash (*Fraxinus pennsylvanica* Marsh, and *F. americana* L.), red maple (*Acer rubrum* L.), silver maple (*A. saccharinum* L.), box elder (*A. negundo* L.), oak (*Quercus* spp.), willow (*Salix* sp.), black cherry (*Prunus serotina* Ehrh.), black walnut (*Juglans nigra* L.), cottonwood (*Populus deltoides* Bartr. Ex Marsh), basswood (*Tilia america* L.), hawthorn (*Crataegus* sp.), prickly ash (*Xanthoxylum americanum* L.), and a few evergreen trees such as pine (*Pinus* spp.). Although there were notable differences in tree species composition, abundance, tree basal area, and tree DBH (diameter at the breast height, ≈1.5 m above the ground) among the four study sites, these characteristics were similar between release and non-release plots within sites.

2.2. Deployment of sentinel EAB eggs to measure parasitism by field-released O. agrili

Within each plot, 10 (2008) or five (2009) green ash trees (*F. pennsylvanica*) without apparent symptoms of EAB infestation (e.g., bark splits, exit holes, epicormic growth, or woodpecker holes) were selected for deployment of sentinel EAB eggs to measure parasitism by field-released *O. agrili*. However, the visual observation of EAB exit holes, bark splits, epicormic growth or woodpecker holes were limited only to the main trunk up to the height of 2 m from the ground. In addition, EAB normally takes two years to complete a generation in MI and symptoms of host tree defense responses (bark split and epicormic growth) and woodpecker predations would not occur until EAB larvae reach late instars in the subsequent growing season. Thus, the observation of no apparent symptoms on the selected trees in our study sties did not necessarily indicate no EAB infestation, but only minimal or light EAB infestation on those selected trees. In fact, the reduction in the number of selected ash trees in 2009 was due to the shortage of suitable ash trees in the study plots because of severe EAB infestation; however, we compensated for this change by doubling the number of sentinel eggs placed on each tree. The ash trees selected for deployment of sentinel EAB eggs in both 2008 and 2009 had DBH values ranging from 7.5 to 25.0 cm among different plots and were separated by a minimum of 5 m and maximum of 300 m within each plot.

Sentinel eggs were produced in the laboratory by providing gravid EAB females with fresh ash twigs (1 cm diameter × 10 cm long). Paper ribbons (≈0.5-cm wide) were wrapped loosely around each ash twig five to six times in a spiral. EAB females readily oviposited under ribbons when twigs were exposed to one or two pairs of EAB in ventilated 500 ml clear plastic cups in an environmental growth chamber (16:8 L:D, 65 ± 10% RH, with daytime and nighttime temperatures cycling between 25 ± 2 and 20 ± 2 °C, respectively). The ash twigs were checked daily by unwrapping the ribbon, and EAB eggs (either single or in small groups) were removed along with the small area of bark (5–7 mm long by 3–5 mm wide) on which eggs were laid.

EAB eggs were deployed in the field by cutting shallow bark flaps (0.2 cm in depth, 0.5 cm wide × 1.0 cm long) on the trunks of the selected ash trees using a utility knife, and inserting the small bark flake (to which the eggs were attached) under the flap. One bark flake, with one or more eggs, was pinned under each bark flap, leaving enough space between the eggs and the flaps to avoid damaging the eggs. Ten bark flaps were created per tree in 2008 and 20 per tree in 2009; half of the eggs were implanted 0.5–1 m and the other half 1.5–2 m above the ground. The locations of the sentinel eggs were indicated by writing numbers next to the bark flaps using weather-resistant ink.

The deployment of sentinel eggs in both the release and control plots at each site was completed in one or two days. Deployments were made in all of the sites over the course of 4 weeks (from June 29 to July 30) in 2008 and 2 weeks (June 16 to June 29) in 2009.

Within 24 h of sentinel egg deployment, female *O. agrili* were released at the base of each selected ash tree in the release sites (10 and 60 per tree in 2008 and 2009, respectively). The sentinel eggs were then left in place for approximately 4 weeks in 2008, but many eggs were lost over that period, possibly due to predation or some other mortality factor. Consequently, the exposure period was shortened to one week in 2009. Because *O. agrili* prefers EAB eggs that are <13 days old (LSB, unpublished data), this change is unlikely to have significantly affected parasitism rates. At the end of the field-exposure period, all the egg-bearing bark flakes were collected, placed individually into 1.5 ml Eppendorf® snap-cap tubes (one bark flake per tube), and brought to the laboratory, where the eggs were incubated for four weeks at 25 ± 2 °C, photoperiod (L:D) of 16:8 h, and ambient RH to recover adult *O. agrili*. After four weeks, the eggs were examined under a dissection (stereo microscope), and assigned to one of the following four categories: (1) hatched – with an exit hole visible on the underside of the egg, (2) unhatched – no exit holes on the egg chorion and no live or dead parasitoid remains such as parasitoid egg, larva, pupa, and/or adult, (3) disappeared – no eggs left or only pieces of egg chorion left on the original egg-bearing bark, and/or (4) parasitized – *O. agrili* exit hole visible on the surface of egg or egg melanized and containing larval and/or pupal stages of *O. agrili*. In both 2008 and 2009, approximately 8% of the egg-bearing bark pieces were accidentally dislodged from the tree, and the eggs on these bark pieces were excluded from the dataset. The parasitism rate of sentinel eggs by *O. agrili* for each tree or study site was calculated as the number of parasitized eggs (with *O. agrili* exit holes or remains) divided by the sum of hatched, unhatched and parasitized eggs. Eggs in the lost and disappeared category were not included in calculation of *Oobius agrili* parasitism because we did not know if those eggs were parasitized or not.

2.3. Survey of egg parasitism in naturally occurring EAB eggs

Approximately 2 weeks after release of *O. agrili*, the main trunk (0.5–2.5 cm above the ground) of each study tree from the parasitoid-release and control plots was searched for naturally occurring EAB eggs for 30 min by one observer or 15 min by two observers. In addition to these ash trees, 10 and five other green ash trees (in 2008 and 2009, respectively) with a similar DBH and within 5–10 m of the study ash trees were also searched for EAB eggs. Those additional green ash trees showed no apparent signs of EAB infestation (bark splits, exit holes, epicormic growth, or woodpecker holes) on the main trunks up to the height of 2 m from the ground line. Naturally occurring eggs were found by carefully looking beneath flakes of bark on the trunks of the trees and collected by removing the pieces of bark ($\approx$0.25 cm^2) to which they were attached. These were individually placed into 1.5 ml Eppendorf® snap-cap tubes, brought back to the laboratory, and incubated (still in the tubes) at 25 ± 3 °C, L:D 16:8 h, and ambient humidity for 5 weeks to recover adult *O. agrili*. At the end of incubation period, unhatched eggs were dissected under a dissecting microscope for any unsuccessful parasitism by *O. agrili* according to method described in Liu et al. (2007). Relative percent parasitism by *O. agrili* was calculated based on total number of eggs collected from all sampled trees.

2.4. Statistical analysis

Likelihood chi-square tests were used to compare the distribution of different fates of sentinel EAB eggs between parasitoid-release and non-release control plots. Percentage EAB egg mortality was calculated for each category such as failure to hatch, parasitism, or disappearance based on the total number of the sentinel eggs deployed on each tree. Percentages in each category of EAB egg mortality were then arcsine-transformed for analysis of variance (ANOVA) with study sites (four in 2008 and three in 2009) as main plots and treatments (parasitoid release vs control) as sub (split) plots. Sampled ash trees within each sub plot were considered as sampling units. For naturally occurring eggs, rates of parasitism were 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 eggs. However, locations of deployed sentinel eggs or naturally occurring eggs on the main trunk of the tree (in the section of two meters above the ground line) were not included in the ANOVA model. Untransformed means and associated standard errors (SE) are used for presentation of the results. All statistical analyses were performed using JMP 8.0.1 (SAS Institute Inc., 2008).

3. Results

3.1. Field-deployed sentinel EAB eggs

In both 2008 and 2009 (Table 1), only 31–40% of the deployed sentinel EAB eggs in both parasitoid-release and control plots hatched; most others either disappeared (37–52%) or failed to hatch (15–24%). While the disappearance of deployed eggs was most likely caused by consumption or removal by predators (e.g., ants, etc.), the unhatched or aborted eggs were possibly due to infertility or other unknown factors (e.g., unsuitable ambient RH or temperature) during the field exposure or laboratory incubation. While no eggs were parasitized by *O. agrili* in any control plots in either 2008 or 2009, 0.8 and 0.9% of the eggs (after excluding eggs disappeared) were parasitized by *O. agrili* in the parasitoid-release plots in 2008 and 2009, respectively. The distribution of different categories of fate of the deployed EAB eggs appeared to be significantly heterogeneous between parasitoid-release and control plots in 2008 (likelihood ratio χ^2 = 9.51, df = 3; P = 0.0232), but was not so in 2009 (likelihood ratio χ^2 = 3.82, df = 3, P = 0.2814). In 2008, more deployed eggs were disappeared (46–52%) than in 2009 (37–40%) in both release and control plots. However, more deployed eggs did not hatch in 2009 (22–24%) than in 2008 (15–16%) in both release and control plots.

There were no significant difference (P > 0.05 for all tests) between parasitoid-release and control plots for any of the egg loss categories in 2008 (Fig. 1A) or in 2009 (Fig. 1B). However, study site had significant effects on all categories of egg mortality in 2008 (for unhatched eggs: F = 3.94, df = 3, 75, P = 0.0115; for disappearance: F = 5.06, df = 3, 75, P = 0.0030; for parasitism: F = 4.68, df = 3, 75; P = 0.0047). In 2009, study site had significant effects on percentages of unhatched or aborted eggs (F = 9.58, df = 2 and 26, P = 0.0008) and disappearance (F = 4.82, df = 2, 26, P = 0.0166), but not on parasitism (F = 0.53, df = 2, 26; P = 0.5942). This indicates that egg mortality due to different biotic and/or abiotic factors varied significantly across different study locations.

3.2. Naturally occurring EAB eggs

The majority (54–66%) of naturally occurring EAB eggs collected from the study plots successfully hatched in both 2008 and 2009 (Table 2), only 23–31% of those collected did not hatch. While no eggs in the control (non-parasitoid release) plots in 2008 nor in 2009 were parasitized by *O. agrili*, 1.1% and 4.2% of the eggs in the parasitoid-release plots were parasitized by *O. agrili*, respectively, in 2008 and 2009. The distributions of hatched, unhatched, and parasitized eggs were not significantly heterogeneous between the parasitoid-release and control plots in 2008 (likelihood ratio χ^2 = 4.73, df = 2, P = 0.0936), but were heterogeneous in 2009 (likelihood ratio χ^2 = 26.84, df = 2, P < 0.0001).

No significant differences (P > 0.05 for all tests) were detected between parasitoid release and control plots in percentage of unhatched eggs and parasitism by *O. agrili* in 2008 (Fig. 2A) or in 2009 (Fig. 2B). Study site had a significant effect on the percentage of unhatched eggs in 2008 (F = 4.5187, df = 3, 52, P = 0.0068), but not in 2009. In both years, there were no significant effects of study site on percent parasitism by *O. agrili*.

4. Discussion

Our results show that both deploying sentinel eggs and collecting naturally occurring eggs can detect parasitism of EAB eggs by *O. agrili*, which was bound to be low for such a recently released biocontrol agent. The failure to detect parasitism by *O. agrili* in any of the control plots also indicates that dispersal or movement by this

Table 1
Distribution of different fates of sentinel EAB egg when deployed on the trunk of selected ash trees in parasitoid release and control plots in 2008 and 2009.

Year of egg deployment	Fates of deployed eggs	Release plots	Control plots
2008	Total number of eggs deployed: *N*	509	419
	Parasitized by *Oobius agrili*: count (%)	2 (0.8)	0 (0.0)
	Hatched to larvae: count (%)	156 (30.6)	164 (39.1)
	Unhatched (dead): count (%)	85 (16.7)	61 (14.6)
	Disappeared in the field: count (%)	266 (52.3)	194 (46.3)
Homogeneity in fates between release and control plots: $\chi^2 = 9.51$, df = 3; $P = 0.0232$			
2009	Total number of eggs deployed: *N*	353	315
	Parasitized by *Oobius agrili*: count (%)	2 (0.9)	0 (0)
	Hatched to EAB larvae: count (%)	143 (40.5)	115 (36.5)
	Unhatched (dead): count (%)	77 (21.8)	75 (23.8)
	Disappeared in the field: count (%)	131 (37.1)	125 (39.7)
Homogeneity in fates between release and control plots $\chi^2 = 3.82$, df = 3, $P = 0.2814$			

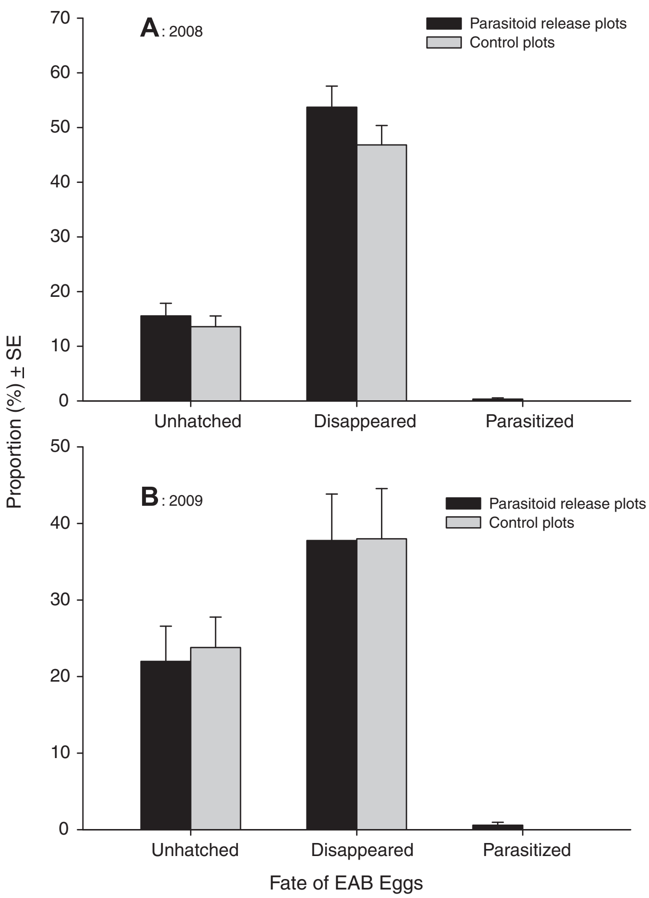

Fig. 1. Comparison of different categories losses of field-exposed sentinel EAB eggs in parasitoid-release and non-release control plots: (A) eggs deployed in 2008, and (B) eggs deployed in 2009.

egg parasitoids is very limited as the distance between control and release plots was less than 1 km. To enhance the spread and establishment of this newly introduced biological control agent, future release programs should include the largest number of release points as possible.

Results from this study showed that deploying sentinel eggs is no more effective at detecting parasitism than collecting naturally occurring eggs. However, the sentinel egg method provides a more accurate estimate of the relative contribution of parasitism by *O. agrili* to total egg mortality relative to other factors. For example, because EAB eggs laid on the surface of ash sticks or logs in the laboratory do not easily fall off the bark surface, the disappearance of many sentinel eggs in this study can likely be attributed to complete consumption by predators such as ants, which were frequently observed on the bark surface during this study. Predation on eggs could be a major mortality factor affecting the establishment of released *O. agrili*, especially since residence time of parasitized eggs is often longer than that of unparasitized eggs (LSB, unpublished data). Such losses would not be noticed by an observer searching for naturally occurring eggs. In contrast to collection of naturally occurring eggs, however, the use of sentinel eggs has certain disadvantages as well. For example, deploying sentinel eggs is more time consuming when considering the laboratory work required for producing EAB eggs for field deployment.

Although we cannot recommend adopting the method for the sole purpose of parasitoid detection at release sites, deploying sentinel eggs may, however, be an important method by which to answer specific questions regarding the biology of *O. agrili* or in assessing this species' relative contribution to EAB egg mortality. The method may also prove useful in other systems involving egg parasitoids of wood-boring insects such as longhorned beetles (Cerambycidae) (e.g., Hanks et al., 1996).

Considering the number of the parasitoids released in both 2008 (10 females per tree or 100 females per site) and 2009 (60 females per tree or 300 females per site), and given that there has been less than two years for released parasitoids to build up

Table 2
Distribution of different fates of naturally occurring EAB eggs observed on green ash trees in parasitoid release and control plots in 2008 and 2009.

Year of observation	Fates of observed eggs	Release plots	Control plots
2008	Total number of eggs observed: *N*	175	123
	Parasitized by *Oobius agrili*: count (%)	2 (1.1)	0 (0.0)
	Hatched to larvae: count (%)	119 (68.0)	95 (77.2)
	Unhatched (dead): count (%)	54 (30.9)	28 (22.8)
Homogeneity in fates between release and control plots: $\chi^2 = 4.73$, df = 2, $P = 0.0936$			
2009	Total number of eggs deployed: *N*	261	420
	Parasitized by *Oobius agrili*: count (%)	11 (4.2)	0 (0.0)
	Hatched to EAB larvae: count (%)	141 (54.0)	275 (65.5)
	Unhatched (dead): count (%)	109 (41.8)	145 (34.5)
Homogeneity in fates between release and control plots: $\chi^2 = 26.84$, df = 2, $P < 0.0001$			

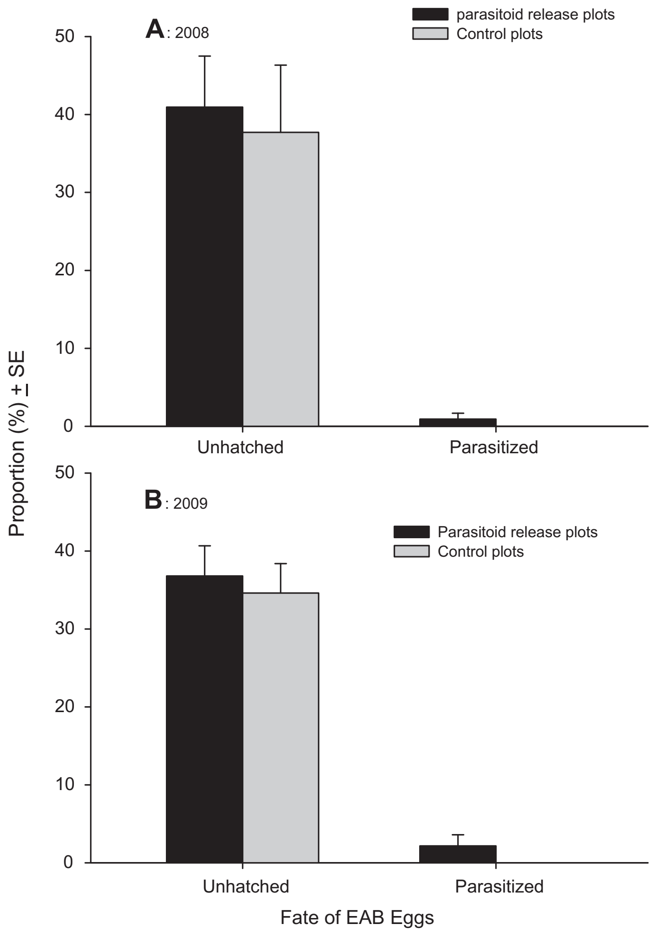

Fig. 2. Comparison of different categories losses of naturally occurring EAB eggs in parasitoid-release and non-release control plots: (A) eggs deployed in 2008, and (B) eggs deployed in 2009. (A) Eggs collected in 2008, and (B) eggs collected in 2009.

populations, it is not surprising that the parasitism rate in both sentinel or naturally occurring EAB eggs (1.1–4.2%) by *O. agrili* in this study was considerably lower than parasitism (>50%) reported from China (Liu et al., 2007). It typically takes several years, for newly introduced biocontrol agents to exert significant impacts on the targeted pests following introduction (e.g., Van Driesche et al., 1998). In fact, the higher rate (4.2%) of parasitism by *O. agrili* in naturally occurring EAB eggs in 2009 as compared to 2008 (1.1%) suggests that the impact of *O. agrili* is likely to increase with time as more releases are made and the species' population density increases.

Considering the rate of parasitism by *O. agrili* in our study, it is not surprising that no statistical differences in parasitism between parasitoid release and control plots were detected between the parasitoid release and control plots or among different study sites in both 2008 and 2009. However, other abundant categories of mortality (failure to hatch and/or disappearance) varied significantly across different study sites in both 2008 and 2009. How the variation in other categories of egg mortality (e.g., predation on eggs) across different study sites would affect the parasitism and establishment of *O agrili* needs to be further investigated for accurately measuring the impact of the parasitoid on EAB egg densities.

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References

Bauer, L.S., Liu, H.P., 2006. *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. 2006 October 29-November 2, Cincinnatti, OH. FHTET 2007-04. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV, pp. 63–64.

Bauer, L.S., Liu, H.P., Gould, J., 2008. Progress on biological control of the emerald ash borer in North America. In: Mastro, V., Lance, D., Reardon, R., Parra, G., (comps.), Emerald Ash Borer Research and Development Meeting; 2007 October 23–24; Pittsburgh, PA. FHTET 2008-07. US Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV, pp. 56–58.

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, 2009 January 13–16, Annapolis, MD. Gen. Tech. Rep. NRS-P-51. USDA Forest Service Northern Research Station, New Town Square, PA, pp. 7–8.

Canadian Food Inspection Agency, 2010. Emerald Ash Borer – *Agrilus planipennis*. 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.

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

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

Hanks, L.M., Paine, T.D., Millar, J.G., 1996. A tiny wasp comes to the aid of California's Eucalyptus trees. California Agriculture 50, 14–16.

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 US 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 US Department of Agriculture interagency research forum on gypsy moth and other invasive species 2006; Gen. Tech. Rep. NRS-P-10. US Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA, p. 66.

Liu, H., Bauer, L.S., Gao, R., Zhao, T., 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., Bauer, L.S., Miller, D.L., Zhao, T., Gao, R., 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.

Michigan State University, 2010. Emerald ash borer information. http://emeraldashborer.info/.

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.

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

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.

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.