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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