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EVALUATING A NEW METHOD FOR MONITORING THE FIELD ESTABLISHMENT AND PARASITISM OF *OObIUS AGRILI* (HYMENOPTERA: ENCYRTIDAE), AN EGG PARASITOID OF EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE)

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Biological control is now an important part of management approaches attempting to both suppress populations of emerald ash borer (EAB), *Agilus planipennis* Fairmaire (Coleoptera: Buprestidae), and slow the spread of this invasive pest. In addition to 2 larval parasitoids, 1 species of egg parasitoid, *Oobius agrili* Zhang & Huang (Hymenoptera: Encyrtidae), has thus far been released in North America as a biological control agent for EAB (Duan et al. 2011, 2012). In the native range of EAB (northeast Asia), parasitism by *O. agrili* can account for 50-60% of egg mortality (Liu et al. 2007), and reducing densities of viable eggs has the potential to limit larval feeding damage to ash (*Fraxinus* spp.) in North America.

Several methods have been developed to monitor parasitism of EAB eggs by *O. agrili*, including searching under bark for EAB eggs, and using sentinel eggs and egg-sentinel logs (ESLs) (Duan et al. 2011, 2012). Although these methods have had some success at detecting *O. agrili* establishment, all have drawbacks. For example, searching for naturally occurring EAB eggs can be time consuming and risks overlooking eggs, whereas sentinel eggs and ESLs are labor-intensive to produce and can suffer from high predation rates. Subsequently, the high numbers of eggs potentially lost to predators could be reducing our ability to effectively recover parasitoid adults and detect parasitism.

In the present study, our objectives were to develop a new method for monitoring *O. agrili* establishment and parasitism that would be simple to replicate, and would reduce predation on EAB eggs by predators without interfering with parasitism. Toward that end we conducted 2 experiments: a laboratory experiment where we assessed the effectiveness of 2 different sized mesh screens on egg parasitism, and a field experiment where we used the same 2 types of mesh screen to determine how effective they were at reducing predation.

EAB eggs used in our experiments were laid by gravid mated females in the laboratory onto a

substrate of coffee filter paper, according to standard rearing procedures (e.g., Duan et al. 2013). The filter paper with EAB eggs was then cut into triangular strips each containing 5, 10 or 25 eggs for use in both experiments. For our laboratory experiment 25 EAB eggs (on 1 filter paper strip) were placed into ventilated 250 mL plastic jars. Treatments consisted of a control where eggs were left completely exposed (i.e., with no mesh), and 2 other treatments where the eggs were inserted into 10 cm × 10 cm envelopes constructed from either 0.5 mm or 1 mm nylon mesh (Fig. 1), with 5 replicates of each treatment. These egg-sentinel envelopes (ESEs) were stapled closed, and 5 adult female *O. agrili* were released into each jar where they remained for 2 days. Eggs were then incubated in an environmentally controlled chamber (25 °C, 65% RH, 16:8 h L:D photoperiod for approximately 21 days) and scored for parasitism based on the emergence of adult wasps as well as discoloration (darkening) of the parasitized eggs in case the parasitoid did not emerge.

Our field experiment was conducted in a natural stand of green ash, *Fraxinus pennsylvanica* Marshall (Oleaceae), in the summer of 2012 at 1 site in northwest Charles County, Maryland. Field experiment treatments consisted of 10 cm × 10 cm mesh ESEs (mesh either 0.5 mm or 1 mm wide), each containing 10 EAB eggs, and controls where the eggs on 1 piece of filter paper were completely exposed, with 4 replicates of each treatment on each of 5 green ash trees. Additionally, we used a thin, transparent plastic screen to cover half of the eggs on each tree, as an extra form of protection from weather and predators. ESEs were placed approximately 1.5 m above ground, and on the same day 30 adult female *O. agrili* were released directly onto each study tree (below the ESEs). The eggs were then left for 2 weeks before being collected and returned to the laboratory to be examined for evidence of parasitism and predation (e.g., jagged chewing marks or loss of entire eggs).

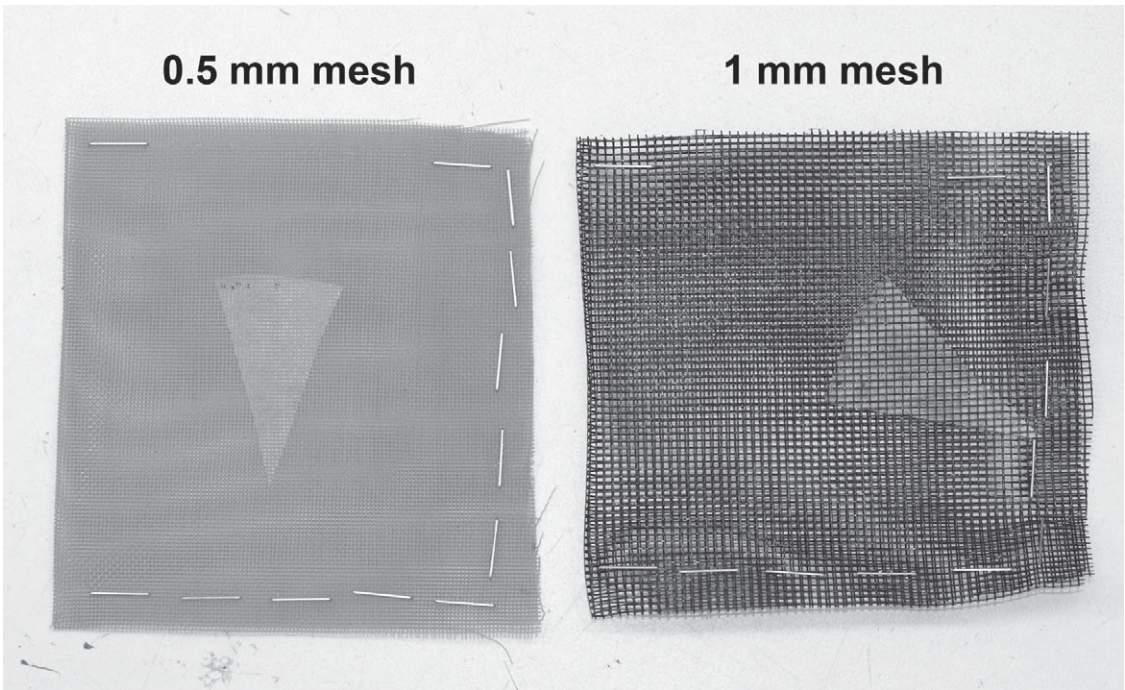


Fig. 1. Photograph of the design of the egg-sentinel envelopes (ESEs) with both types of mesh and example eggs inserted. The tops of each ESE were stapled closed before starting any experiments.

Data were analyzed using R 3.0.2 (R Core Team 2013). We examined how the screening treatment affected the number of eggs parasitized in the laboratory, and then how it affected the number of eggs parasitized and eaten in the field. Additionally, in the field experiment we tested for effects of tree and plastic screening protection. Using the package *nlme*, we constructed generalized linear models with a Poisson error distribution to compare models, and significance was tested using χ^2 tests based on log-likelihood ratios. Goodness-of-fit to a Poisson distribution was tested using χ^2 tests based on residual deviance and degrees of freedom; all $P > 0.05$ indicating that the data fit this distribution. Tukey's HSD test was used to conduct post-hoc comparisons when significant main effects were detected.

In the laboratory parasitism was highest on exposed eggs (mean percent \pm SE = $70.4\% \pm 7.7$), followed by 1 mm mesh ($64\% \pm 11.3$) and then 0.5 mm mesh ($45.6\% \pm 11.6$), and there was a significant effect of mesh size on the number of eggs parasitized ($\chi^2 = 7.17$, $df = 2$, $P < 0.001$). Additionally, there was a significant difference in parasitism between the 0.5 mm mesh and the completely exposed treatments (Tukey's HSD: $P = 0.029$) (Fig. 2a).

For the field experiment, parasitism across all treatments was considerably lower in comparison to the laboratory experiment (Fig. 2b). We found no significant effect of mesh size ($\chi^2 = 4.38$, $df = 2$, $P = 0.112$), plastic screening protection ($\chi^2 = 1.01$, $df = 1$, $P = 0.315$), or tree ($\chi^2 = 2.44$, $df = 1$, $P = 0.118$) on the

number of eggs parasitized. However, there was a significant effect of mesh size on the number of eggs eaten ($\chi^2 = 187.32$, $df = 2$, $P < 0.001$), but protection ($\chi^2 = 0.20$, $df = 1$, $P = 0.655$) and tree ($\chi^2 = 2.86$, $df = 1$, $P = 0.091$) were not significant. There was almost no predation on eggs in ESEs with the 0.5 mm mesh, and there were significant differences in predation between exposed eggs and 1 mm mesh (Tukey's HSD: $P < 0.001$), exposed eggs and 0.5 mm mesh (Tukey's HSD: $P < 0.001$), and 1 mm mesh and 0.5 mm mesh (Tukey's HSD: $P < 0.001$) (Fig. 2c).

Our results provide support for using ESEs made of 0.5 mm mesh as a method to effectively protect EAB eggs from predation by ants and other predators, while also minimizing interference with parasitism by *O. agrili* on EAB eggs in the field. Although the 0.5 mm mesh appears to reduce parasitism under laboratory conditions, parasitism was low across all of our treatments in the field ($\sim 5\%$ for all treatments). This could be a result of the number of parasitoids we released, and it would be interesting to test this screening method using greater numbers of parasitoids. Perhaps more importantly, future work should examine how effective different types of mesh ESEs can be at detecting naturally occurring parasitism directly alongside other previously employed methods such as ESLs.

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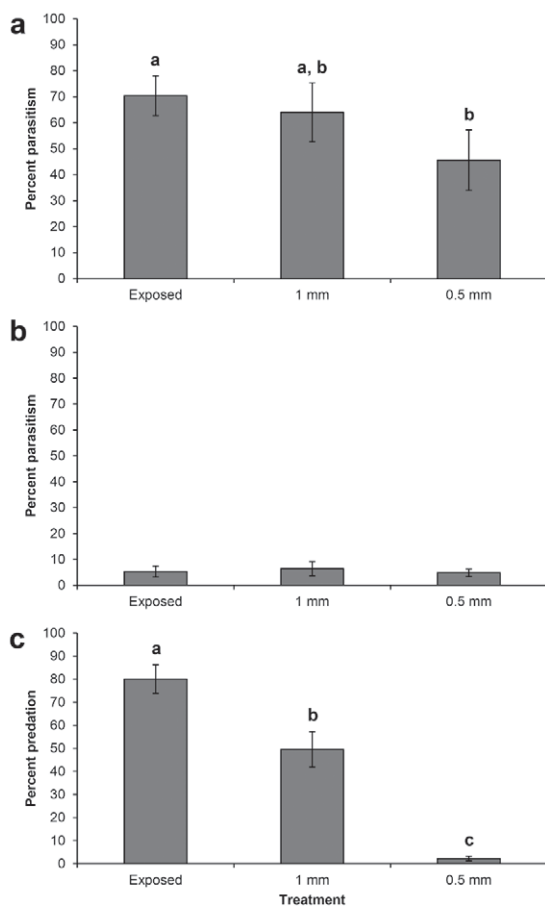


Fig. 2. Relationships between mesh treatment and the percent parasitism of EAB eggs in a) the laboratory and b) the field, and c) the percent predation of eggs in the field (all \pm SE). Different lowercase letters represent treatments that were significantly different from each other.

tance with this study. We also are grateful to Doug Luster and Roger Fuester (USDA-ARS) for providing helpful comments on the manuscript.

SUMMARY

We tested 10 cm \times 10 cm mesh egg-sentinel envelopes (ESEs) containing emerald ash borer eggs on filter paper, and assessed how 2 different mesh sizes (0.5 mm and 1 mm) affected parasitism by *Oobius agrili* in the laboratory, and parasitism and predation in the field. Mesh size significantly affected parasitism in the laboratory, with the 0.5 mm mesh reducing parasitism by approximately 20% relative to the 1 mm mesh. Parasitism was much lower in the field with no significant difference among treatments, but the 0.5 mm mesh did significantly reduce predation by almost 50% in comparison to the 1 mm mesh. To reduce egg

predation while enabling detectable levels of parasitism by *O. agrili*, we therefore recommend using mesh screen 0.5 mm wide to create ESEs for field deployment.

Key Words: *Agrilus planipennis*, biological control, *Fraxinus pennsylvanica*, invasive species

RESUMEN

En el presente estudio, hicimos sobres de un tamiz de 10 cm \times 10 cm para los huevos centinales (SHC) que tienen huevos del barrenador esmeralda del fresno sobre papel de filtro, y probamos cómo los 2 diferentes tamaños de tamices (0.5 mm y 1 mm) afectó el parasitismo por *Oobius agrili* en el laboratorio, y el parasitismo y la depredación en el campo. El tamaño del tamiz afectó el parasitismo significativamente en el laboratorio, con el tamiz de 0.5 mm reduciendo

el parasitismo aproximadamente un 20% respecto al tamiz de 1 mm. El parasitismo fue mucho menor en el campo, sin diferencias significativas entre los tratamientos, pero el tamiz de 0.5 mm redujó significativamente la depredación de casi un 50% en comparación con el tamiz de 1 mm. Para reducir la depredación de huevos y permitir un nivel detectable de parasitismo de *O. agrili*, por lo tanto, recomendamos el uso de un tamiz de 0.5 mm de ancho para hacer los SHC para su uso en el campo.

Palabras Clave: *Agrilus planipennis*, control biológico, *Fraxinus pennsylvanica*, especies invasoras

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