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Gypsy Moth (Lepidoptera: Lymantriidae) Sterile Egg Mass Augmentation Increases *Entomophaga maimaiga* Density[1]

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The fungus *Entomophaga maimaiga* Humber, Shimazu & Soper, is one of the most effective biological control agents of the gypsy moth, *Lymantria dispar* L. It was first recovered from North American gypsy moth populations in 1989 (Andreadis and Weseloh 1990. Proc. Natl. Acad. Sci. USA. 87: 2461-2465). In northeastern North Carolina, the southern edge of the gypsy moth range in North America, there are sites where *E. maimaiga* has been established for up to 9 yrs (Gillock and Hain 2002. J. Entomol. Sci. 37: 366-369).

Hajek et al. (1995. Biol. Control. 5: 530-544) expressed concern that the high virulence and high specificity of *E. maimaiga* towards gypsy moth might result in the loss of a minimum host reservoir to maintain infective inoculum in the soil. Although *E. maimaiga* azygospores (i.e., resting spores) may remain infective for up to 6 yrs (Weseloh and Andreadis 1997. J. Invertebr. Pathol. 69: 195-196) the inoculum density may decrease by rainfall dispersal and movement into the soil profile via soil cracks (Hajek et al. 1998. BioContr. 43: 189-200).

This study reports on our attempt to increase *E. maimaiga* azygospore inoculum by increasing the host reservoir with the placement of gypsy moth sterile egg masses. Distribution of sterile egg masses successfully augmented populations of the gypsy moth parasitoid *Cotesia melanoscela* (Ratzburg) in MA in 1987 (Liebhold and Elkinton 1989. Environ. Entomol. 18: 986-995). We received sterile egg masses from the USDA-APHIS Otis Plant Protection Center (Otis Air National Guard Base, MA) on 14 April 2000. These eggs had been made sterile by treating the P1 males with 15 kilorads of radiation during their pupal stage. It was determined by microscopic examination that each gram of egg mass contained approximately 1400 eggs. However, in addition to sterility the F1 generation also exhibited reduced overall fitness, with about 20% expected survival to late instar; therefore, each gram of egg mass effectively contained approximately 280 viable eggs.

Azygospore density around tree bases was measured on 13 March 2000 (before

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the period of larval activity that season; hereafter, "preseason") and on 05 July 2000 (after larval activity had ceased; hereafter, "postseason") to determine any increases in azygospore inoculum in the soil. Our azygospore sampling techniques were those of Hajek and Wheeler (1994. J. Invertebr. Pathol. 64: 71-73), except rather than using a whole azygospore count, our final suspension was concentrated and then azygospores were enumerated using a Bright-Line hemacytometer (Reichert, Buffalo, NY). Although our physical counts could not positively identify viable azygospores, it was assumed that, given a standard proportion of viability, higher total counts would signify higher viable counts.

Our five experimental plots in northeastern North Carolina (Camden Co. and Currituck Co.) had all yielded *E. maimaiga*-infected larvae for at least 3 yrs previous to this study (R. Copeland, NC Depart. Agric., pers. comm). The plots were swamps that experienced periodic flooding, with tree cover of predominantly water oak (*Quercus nigra* L.), sweet gum (*Liquidambar styraciflua* L.), and loblolly pine (*Pinus taeda* L.). The plots were each at least 2 ha in size, and were at least 3.2 km from each other to decrease possible conidial transfer among treatment areas.

Two areas (Camden Co.) were treated with 62 g of sterile egg masses (approximately 17,360 viable sterile eggs) per tree on 20 April 2000. Two other areas (Currituck Co.) received 250 g of sterile egg masses (approximately 70,000 viable sterile eggs) per tree on the same date. At all four sites, egg masses were distributed about the bases of three trees. A control plot in Currituck Co. received no egg masses. The sterile gypsy moth larvae were allowed to hatch and develop normally throughout the season, presumably to become infected with *E. maimaiga* and contribute azygospores to the soil as they died.

No significant defoliation was observed during the course of the experiment. We found that the mean (±SE) number of azygospores per g of dry soil at the control plot was 4000 ± 1000 in preseason and 2900 ± 1500 in postseason, showing a seasonal decrease in azygospore inoculum levels. In Camden Co., the mean (±SE) number of azygospores per g of dry soil was 1800 ± 900 in preseason and 10,900 ± 2000 in postseason. In Currituck Co., the mean (±SD) number of azygospores per g of dry soil was 7000 ± 2000 in preseason and 32,000 ± 4800 in postseason. The azygospore inoculum increased in those areas in which sterile egg masses were added. However, these results cannot be verified statistically because our plots were not chosen at random. The areas receiving the lower levels of egg masses were privately owned, and the grower cooperator would not agree to higher augmentation levels.

Augmentation with gypsy moth sterile egg masses has some drawbacks. The production of sterile egg masses is a costly and time-consuming process, requiring specialized irradiation equipment and rearing facilities, and a lengthy diapause before hatching. This augmentation technique is not useful for immediate gypsy moth control in an outbreak situation, because the azygospores generated require an overwintering period to become infective, and the technique may not be prudent for use in residential areas.

Primary infection initiated by azygospores and secondary infection from conidial transmission are jointly responsible for the production of epizootics, and both are highly dependent upon cool, wet springs for success (Shimazu et al. 1987. Appl. Entomol. Zool. 22: 216-221). However, maintaining high azygospore inoculum levels in the soil may induce earlier epizootics by increasing the chances that a gypsy moth larva will come into contact with a viable and infective azygospore. For this reason, high inoculum levels in the soil are important, even though *E. maimaiga* infection often

ensues from a single azygospore-borne conidia, and no LD50 values are typically associated with *E. maimaiga*. Hajek et al. (2002. J. Invertebr. Pathol. 79: 37-43) found that percent conidial germination was not associated with conidial density on larval cuticle.

Sterile host augmentation would be most useful as part of a long-term gypsy moth management plan. Factors that must be considered in determining its utility include gypsy moth population levels, initial azygospore inoculum in the soil, *E. maimaiga* infection levels, and the presence/absence of other natural enemies of the gypsy moth.

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