N O T E

Entomophaga maimaiga (Entomophthorales: Entomophthoraceae) Infects *Malacosoma americanum* (Lepidoptera: Lasiocampidae) in North Carolina[1]

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Entomophaga maimaiga Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) appears to have a very narrow natural host range infecting primarily *Lymantria dispar* (L.). Lepidoptera is the only susceptible insect order, and Lymantriidae was the only family that showed high levels of infection in a laboratory study (Hajek et al. 1995b. Biol. Contr. 5: 530-544). In a field study (Hajek et al. 1996. Environ. Entomol. 25: 709-721), only two individuals showed infection: one of 318 *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) and one of 96 *Catocala ilia* (Cramer) (Lepidoptera: Noctuidae). Of six species that were infected in the lab study, only one (*M. disstria*) was found infected in the field, and at a much lower level. This confirms that laboratory host range studies are not always reliable indicators of natural host range in the field.

The ability of *E. maimaiga* conidia to infect a group of larvae may be related to larval setation and epicuticular sculpturing. The cuticle can be a barrier to conidial adhesion, host recognition (either thigmotropic or chemical), and/or penetration. Chances of infectivity are decreased in species without pronounced sculpturing and with only primary setae or short secondary setae (Hajek et al. 1995b). The lymantriids (tussock moths) have very dense setae and also show the highest levels of infection. More setae could increase the surface area available for conidial attachment or could possibly increase the relative humidity at the cuticular level, improving the chances for conidial survival (Hajek. 1999. Microbiol. Mol. Biol. Rev. 63: 814-835).

The purpose of this study was to examine the susceptibility in the field of a common forest insect pest when prolonged exposure to *E. maimaiga* occurs. *Malacosoma americanum* (F.) is hairy and appears to be a likely host since earlier work showed *M. disstria* to be a host in both the lab and the field. The study took place in northeastern North Carolina at the southern edge of the North American *L. dispar* infestation.

The study site was in Camden Co., NC, in a swampy area that experiences

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periodic flooding. Its tree cover is predominantly water oak (*Quercus nigra* L.), sweet gum (*Liquidambar styraciflua* L.), and loblolly pine (*Pinus taeda* L.). Gypsy moth has been in northeastern North Carolina since at least 1974 (North Carolina Department of Agriculture and Consumer Services 1999), and *E. maimaiga* was introduced to this site in 1996 in the form of azygospores (resting spores) in soil, transported from Rockbridge Co., VA. Annual records maintained by the North Carolina Department of Agriculture indicate that *E. maimaiga* infection of *L. dispar* has occurred there every year since 1997.

Soil samples were collected at the base of a water oak near the center of the fungus introduction area on 13 March 2000, and were analyzed for azygospore content. This procedure is a modified version of the technique described by Hajek and Wheeler (1994. J. Invertebr. Pathol. 64: 71-73). Briefly, the soil was weighed, dried, and weighed again to determine percent moisture (71%). It was then ground with a mortar and pestle, and 5 g of it was mixed with a detergent solution. This mixture was sonicated for 2 min and then washed through a sieve series. The particles between 20 and 63 µm, including any azygospores in the sample, remained on a 20 µm sieve, and were washed into 50 ml of NaCl. Five ml of this mixture was layered onto the top of a Percoll density gradient that was then centrifuged for 10 min. Bands containing azygospores were extracted, diluted with NaCl, and centrifuged again to pellet the azygospores.

Azygospores in the pelleted portion were counted with a haemocytometer. Ten counts were taken for the soil collected at the tree and were averaged together to find an estimate of the mean (ISE) number of azygospores per g of dry soil (22,500 ± 5700).

The *M. americanum* larvae were field collected as third instars from cherry trees (*Prunus* sp.) in Raleigh, NC, on 27 March 2000, and were reared to fourth and fifth instars on cherry foliage. Eleven cages were constructed from 30 × 30 cm (12″ × 12″) squares of aluminum window screen. Each square was folded in half, and the edges were duct-taped and then stapled to form a 30 × 15 cm cage. Ten larvae were placed into each of 11 cages. The *M. americanum* larvae (approximately 5 fourth instars and 5 fifth instars per cage) were provided with cherry foliage in florist vials. On 17 April 2000, 10 cages were distributed evenly around the base of the water oak, on top of the organic layer of soil, and were pinned to the ground with metal stakes. The eleventh cage remained in the lab as a negative control.

The cages were returned to the lab on 20 April 2000. A 3-d field exposure was considered long enough to acquire *E. maimaiga* infection. A longer exposure would have increased the risk of ant predation, parasitoid attack, and depletion of foliage in the cages. The larvae were placed in 15 × 100 mm Petri dishes (5 larvae per dish). They were provided with fresh cherry foliage daily and were monitored for 10 d or until pupation. Those that died were placed on 1% water agar dishes and held in the dark. These were checked twice daily for 3 d for production of conidia. They were then held for another 7 d, after which they were dissected to check for azygospores.

The high levels of azygospores in the soil (22,500 ± 5700 per g of dry soil) showed the tree to be a suitable experimental site. In addition, many *L. dispar* larval cadavers were collected from trees in this area in the spring and the previous spring and indicated an active *E. maimaiga* population. The soil moisture averaged 71%, due to the swampy nature of the site. Thus, no watering of the soil was necessary to promote germination of azygospores.

Two of 98 *M. americanum,* both fifth instars, died from an entomophthoralean

infection and produced conidia overnight in their Petri dishes. They had been exposed in different cages. After being held on water agar plates in the dark for 7 d, they were found to contain entomophthoralean azygospores. All 10 control larvae survived to pupation.

The conidia and azygospores from the two infected larvae were only identified morphologically. Although they appeared to be *E. maimaiga,* they may have been *E. aulicae,* a closely-related entomopathogen in the same species complex. *Entomophaga maimaiga* and *E. aulicae* are morphologically indistinguishable, although *E. aulicae* cannot infect *L. dispar.*

Future studies should include greater replications of this treatment. This study would also have had greater value if *L. dispar* larvae had been present in the lab to expose the sporulating *M. americanum* larvae to. This could have provided a definitive *E. maimaiga* species determination, as only *E. maimaiga* can infect *L. dispar.* Simply caging *L. dispar* larvae in the field adjacent to the *M. americanum* larvae would not have proven *E. maimaiga* infection in the *M. americanum* larvae, because there is no guarantee that the two species of larvae would be infected by the same species of entomopathogen. However, simultaneously caging *L. dispar* larvae in the field could confirm a high level of *E. maimaiga* azygospore germination, thereby eliminating the need for the lengthy soil analysis.

For *E. maimaiga* to infect hosts other than *L. dispar,* these hosts would have to exhibit the same seasonal, temporal, and spatial activity as *L. dispar* in order to encounter *E. maimaiga* (Hajek et al. 1995b). *Malacosoma americanum* satisfies these requirements. Seasonally, *M. americanum* larvae are present at the same time of year as *L. dispar* larvae and *E. maimaiga.* Temporally, they spend the day inside their protective tents, emerging at night and in rainy weather to feed (Leathers and Gupta 1993. J. Invertebr. Pathol. 61:217-219). This causes them to be present in the environment at the same time that *E. maimaiga* conidia are most likely to be discharged (Hajek and Soper 1992. Environ. Entomol. 21:129-135).

Spatially, late-instar *M. americanum* larvae may encounter azygospores in the soil when crossing the ground to a new host tree, or when wandering in search of pupation sites. Furthermore, larval aggregation in tents would increase the opportunity for *E. maimaiga* conidial transmission from an infected individual to an entire colony. Any contagious biological control agent should be particularly effective against *M. americanum* due to this aggregation tendency (Leathers and Gupta 1993).

Despite the fact that *M. americanum* appears to be a fitting candidate for *E. maimaiga* infection, and despite the fact that the experimental site had high azygospore densities in the soil, approximately 98% of the *M. americanum* larvae exposed in this study did not succumb to any entomophthoralean infection. This supports previous studies that found a narrow host range for *E. maimaiga* (Hajek et al. 1995b, Hajek et al. 1996).

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