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Spread of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) from Initial Introduction Areas in North Carolina[1]

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Entomophaga maimaiga Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) is a fungal pathogen of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). It is considered unusual among insect pathogens due to its rapid rate of spread throughout its host range in North America (Hajek et al., 1995, Am. Entomol. 41: 31-42; Hajek, 1999, Microbiol. Mol. Biol. Rev. 63: 814-835). In the past decade, introductions of *E. maimaiga* into leading-edge areas, followed by extensive natural spread of the fungus from these introduction sites, resulted in widespread *E. maimaiga* establishment in much of the *L. dispar* range (Smitley et al., 1995, Environ. Entomol. 24: 1685-1695; Hajek and Elkinton, 1992, USDA Interagency Gypsy Moth Research Forum 1992, General Technical Report NE-170, 23; Hajek et al., 1996, Environ. Entomol. 25: 1235-1247).

The study reported here documents the natural spread of *E. maimaiga* from three sites in eastern North Carolina where it was intentionally introduced in 1992-1999. These sites are at the southern edge of the *L. dispar* range in North America, and are all swampy areas that experience periodic flooding.

Two sites, called Old Trap and New Old Trap, are in Camden Co., in the northeastern corner of North Carolina. The third, called Devil's Gut, is in Martin Co., in east-central North Carolina. *Entomophaga maimaiga* was introduced to Old Trap by North Carolina Department of Agriculture and Consumer Services (NCDA&CS) personnel in 1992, as azygospores in soil from Sussex Co., New Jersey. The soil was distributed around the bases of designated "introduction area" trees, coated onto burlap bands that were then attached to the trees, and exposed to released neonate larvae the following spring. The fungus was similarly introduced to New Old Trap, from Rockbridge Co., Virginia in 1996. It was introduced to Devil's Gut as azygospores in soil and in *E. maimaiga*-killed *L. dispar* larval cadavers, from Currituck Co., North Carolina, in 1999.

Annual records maintained by the NCDA&CS indicate *E. maimaiga* infection occurring at Old Trap since 1994, at New Old Trap since 1997, and at Devil's Gut since

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1999. Tree cover is predominantly water oak (*Quercus nigra* L.), sweet gum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.) at Old Trap and New Old Trap, and oak, sweet gum, pine and cypress (*Taxodium distichum* [L.] Rich.) at Devil's Gut.

At the Old Trap and New Old Trap sites, three trees, approximately 30 m from each other in a triangle formation, were selected at the *E. maimaiga* introduction area and were banded with burlap on 13 March 2000. At each site, a 100-m transect was extended from the introduction area to the north, east, and northeast. Along each transect, a tree was banded with burlap at every 25-m mark. A northeastern zone was chosen for survey because the prevailing winds at both sites were from the southwest, so spread of *E. maimaiga* by airborne conidia was expected to be to the northeast. One tree, 25 m to the southwest of each introduction area, was also banded with burlap to check for possible *E. maimaiga* spread in that direction. Species and diameter at breast height (DBH) was noted for each tree (Gillock, 2001, M. S. Thesis, North Carolina State University, Raleigh).

On 29 March 2000, two trees approximately 50 m from each other, at the *E. maimaiga* introduction area at Devil's Gut were banded with burlap. A 30 m transect to the northeast of each tree was extended, and a tree at every 10 m along each transect was banded with burlap. Species was noted for each tree (Gillock 2001). Although *E. maimaiga* spread could have been expected beyond 100 m and 30 m, these transect lengths were chosen because this was the first reported study on spread of *E. maimaiga* in this region. Its purpose was to verify spread to at least these distances. Future studies may require longer transects.

On 13 March 2000, soil samples were collected at the bases of the 3 introduction area trees banded at Old Trap and New Old Trap, as well as from 3 trees (results averaged together) near the 100 m mark on the northeast transect at both sites. On 29 March 2000, soil was collected at the bases of both of the introduction area trees banded at Devil's Gut. No soil collection was made at the 30-m mark because *E. maimaiga* spread was not expected at this distance following the previous year's introduction.

The soil samples were analyzed for azygospore content by a modified version of a standard technique (Hajek and Wheeler, 1994, J. Invertebr. Pathol. 64: 71-73). Briefly, the soil was weighed, dried, and weighed again to determine percent moisture. 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 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 which was then centrifuged for 10 min. Bands containing any azygospores were extracted, diluted with NaCl, and centrifuged again to pellet the azygospores.

Samples of the pelleted portion were counted on a hemocytometer, with 10 counts taken for each tree's soil samples. The 10 counts were averaged together to find the mean number of azygospores per g of dry soil at each tree sampled (Table 1). Soil sampling and analysis was done to give an additional indication of *E. maimaiga* presence, and to determine if azygospore presence was always associated with larval infection.

The mean (±SE) azygospore density at the three introduction trees was 4900 (±1600) azygospores per g of dry soil at Old Trap, 9400 (±2400) azygospores per g of dry soil at New Old Trap, and 8700 (±2700) azygospores per g of dry soil at Devil's Gut. No correlation of azygospore density with percent soil moisture was found.

Table 1. *E. maimaiga* azygospore density at each tree sampled

Site and tree number	Mean number of azygospores (± SE) per g of dry soil
Old Trap # 1	4400 ± 2300
Old Trap # 2	8500 ± 3800
Old Trap # 3	1500 ± 1500
Old Trap 100 m NE	4600 ± 1600
New Old Trap # 1	22500 ± 5700
New Old Trap # 2	1600 ± 1600
New Old Trap # 3	6700 ± 3000
New Old Trap 100 m NE	3700 ± 1600
Devil's Gut # 1	11800 ± 4100
Devil's Gut # 2	4800 ± 2400

Egg hatch of *L. dispar* at these sites occurred from approximately mid-April to the beginning of May 2000, with the larval season ending near the beginning of June 2000. On 3 May, 17 May, 24 May, and 31 May 2000, *L. dispar* larvae were collected from the banded trees at Old Trap and New Old Trap. All larvae found on or under the burlap bands or on the foliage of banded trees were placed in individual diet cups (Southland Products, Lake Village, AR) and returned to the lab. Larvae were similarly collected from the banded trees at Devil's Gut on 17 May, 22 May, and 8 June 2000. Larvae were held in their diet cups in the lab for 10 d or until death or pupation occurred. Those that died were placed in the dark and were checked twice daily for 3 d for production of conidia. The cadavers were held an additional 10 d and then dissected to check for azygospores. The presence of either conidia or azygospores confirmed *E. maimaiga* infection.

A total of 285 *L. dispar* larvae were collected during the study period. Twenty-one were collected at Old Trap, 158 were collected at New Old Trap, and 106 were collected at Devil's Gut. Thirty-eight larvae, all collected at New Old Trap, died of *E. maimaiga* infection. Seven were collected on 24 May 2000, and 31 were collected on 31 May 2000. This represents a 24% *E. maimaiga* infection rate at New Old Trap. The number of larvae and percent infection found at each banded tree at New Old Trap is shown in Table 2. No infected larvae were found at Old Trap or Devil's Gut on any of the sampling dates.

The infection seen at New Old Trap illustrates the distance that *E. maimaiga* has spread here in the past 4 yrs. Infected larvae were found as far as 100 m east (seven out of eight larvae collected there), and 75 m north (four out of 31 larvae collected there) of the *E. maimaiga* introduction area. However, most of the infected larvae were still found at the introduction trees (23 out of 81 larvae collected at those trees). The fact that infection was seen at a tree 100 m from the introduction area shows that *E. maimaiga* has spread at least to, and very likely beyond, this distance. The presence of azygospores in the soil at the end of the 100 m northeastern transect support

Table 2. Number of *L. dispar* larvae found and % *E. maimaiga* infection observed at each banded tree at New Old Trap

Tree Location	No. larvae found	% of larvae infected
Introduction tree 1	27	22
Introduction tree 2	47	36
Introduction tree 3	7	0
25 m north	4	50
50 m north	18	6
75 m north	31	13
100 m north	0	0
25 m east	0	0
50 m east	0	0
75 m east	7	0
100 m east	8	88
25 m northeast	7	14
50 m northeast	0	0
75 m northeast	1	0
100 m northeast	0	0
25 m southwest	1	0

this assumption, as do experiments by others that show *E. maimaiga* spread rates of up to one km in a single season (Hajek et al., 1996, Environ. Entomol. 25: 1235-1247).

The lack of infection seen at the Old Trap and Devil's Gut sites cannot be attributed to the environmental conditions measured, because soil moisture, the factor most influencing *E. maimaiga* success, was roughly equivalent at all three sites (74 ± 3.9% at Old Trap, 69 ± 7.3% at New Old Trap, and 62 ± 3.5% at Devil's Gut). Furthermore, infected larvae had been recovered from all three sites in previous years, and all of the soil samples collected showed *E. maimaiga* azygospore levels sufficient to cause infection in *L. dispar* larvae (Table 1).

Entomophaga maimaiga was introduced at New Old Trap 4 yrs, at Old Trap 8 yrs, and at Devil's Gut 1 yr prior to this study, It may be that too few *L. dispar* larvae remain at Old Trap to support high infection levels, while at New Old Trap, the infection dynamic is still active. At Devil's Gut, *E. maimaiga* may not yet be broadly distributed enough to cause widespread infection. At all three sites, *L. dispar* population density had declined below the point where egg mass surveys were routinely performed. A great deal remains to be discovered about *E. maimaiga* infection dynamics, including the relationship between *L. dispar* population cycles and azygospore levels, and threshold azygospore levels required for infection.

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